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THE FUNCTION AND STRUCTURE OF THE PARENCHYMA SHEATH PLASTIDS OF THE MAIZE LEAF

M. M. RHOADES AND ALCIDES CARVALHO[1]

In connection with a genetic study of chlorophyll variegation in maize (Rhoades 1943) it was necessary to prepare sectioned material of the leaf. When these slides were examined, our attention was caught by the unusual plastids of the single-layered parenchyma or bundle sheath cells surrounding the vascular bundles. A survey of the literature disclosed no account of these plastids which agreed with our conception of their structure and function; so a brief account has been prepared.

Many grasses have two cell layers surrounding the vascular bundles, while others have a single layer. Schwendener (1890) designated the outer layer as the parenchyma sheath and the inner layer, whose inner walls are often thickened, as the mestome sheath. The parenchyma sheath cells of some grasses contain chloroplasts, while these cells of other grasses are colorless. The members of the subfamily Pooideae have the vascular bundles enclosed in a thick-walled mestome sheath surrounded by a parenchyma sheath, while in the subfamily Panicoideae there is only a parenchyma sheath (Arber 1934). That the cells of the parenchyma sheath of many grasses contain chloroplasts has long been known, and their presence led Haberlandt (1914) to suggest that the green parenchyma sheath cells might have an undiscovered function in addition to their acting both as the efferent tissue and as an unimportant addition to the chlorophyll-apparatus of the plant. Although this question posed by Haberlandt has been answered in part, it is the purpose of this paper to present further studies on the structure and function of the chloroplasts in the parenchyma sheath cells of the maize leaf.

Schwendener (1890) and Strasburger (1891) reported that the parenchyma sheath enclosing the vascular bundle of the maize leaf was chlorophyll-bearing. Kiesselbach (1916) observed that the parenchyma sheath cells of maize contained chloroplasts larger than those of the mesophyll cells. He further stated that these plastids "assumed an abnormal elongated shape and grouped themselves in an unnatural crescent arrangement about the outer edge of the cells" when subjected to a chrome-acetic fixing agent. Weatherwax (1923) mentions the large prominent chloroplasts of the parenchyma sheath but adds nothing to Kiesselbach's observations.

[1] Rockefeller Foundation Fellow at Columbia University.

Eames and MacDaniels (1925) figure a cross section of a corn leaf which gives a misleading picture of the parenchyma sheath plastids. They also identify the parenchyma sheath of grasses as the mestome sheath, which is contrary to the terminology of other investigators. Avery (1930) also figured and briefly described the parenchyma sheath of the leaf bundles of maize and stated: "The vascular bundles are surrounded by a chlorophyll-bearing bundle sheath. The chloroplasts of the bundle sheath cells differ from those of other cells of the leaf." Arber (1934) figures a cross section of a leaf bundle of maize showing the parenchyma sheath but makes no statement about the plastids. Sharman (1942) and Esau (1943) have made careful studies on the ontogeny of the vascular bundles of maize. Esau noted that the parenchyma sheath cells contained abundant large chloroplasts, while Sharman made no mention of the plastids. The papers cited above may be summarized, in so far as they concern the parenchyma sheath, as agreeing only that the single layer of cells comprising the parenchyma sheath around the vascular bundles in the maize leaf contains numerous chloroplasts larger than those of the mesophyll cells.

Zirkle (1929) in an intensive study of the plastids of maize recognized that the cells of the bundle sheath contained specialized chloroplasts concerned with starch storage. He believed that the smaller plastids of the mesophyll had central vacuoles containing starch when conditions were favorable for photosynthesis. Each plastid of the bundle sheath cells was thought to contain in the plastid vacuole a centrally located starch grain. According to Zirkle, the surfaces of these plastids possess pores leading to their central vacuoles. When the plastids were oriented on their flat surfaces, the pores appeared round, but when the plastids were viewed from the side they appeared as slits. He concludes that the pores are concentrated on the flatter surfaces of the plastid and lead to the vacuole but do not point to the plastid's center. We believe that Zirkle has misinterpreted several important structures. Further reference to his account of these plastids will be made later.

Weier (1932), in a paper dealing with the possible homology of the plastids with the Golgi zone, figured a cell from a maize leaf which unquestionably came from the parenchyma sheath, although Weier does not so state. He found that the plastids of this cell contained "cavities" filled with starch. He says nothing of the kinds of cholorplasts found in the mesophyll and bundle sheath cells.

STRUCTURE OF THE BUNDLE SHEATH PLASTID

A brief survey of the voluminous but controversial literature on plastid structure reveals that their fixation images may differ greatly from their appearance in living cells. Since most of our observations were made on

fixed material, it is clear that our comments on the structure of the parenchyma sheath plastids must remain of a tentative nature until a more thorough study has been made. However, some of the grosser features are clearly revealed, and it is with these that we shall concern ourselves.

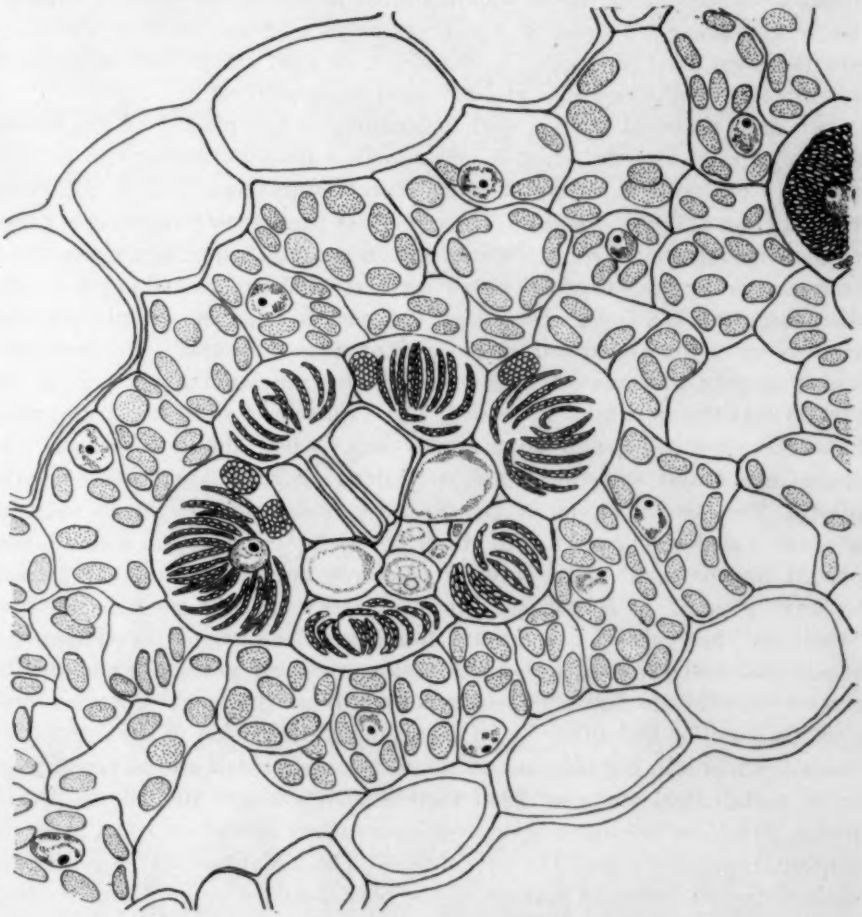


FIG. 1. Cross section of a maize leaf showing single-layered parenchyma sheath enclosing vascular bundle containing flattened ellipsoidal starch-containing plastids. Most of the plastids in the sheath cells are so oriented that a cross section of the leaf presents them in side view, but a few are shown in face view. The sheath cells contained many more plastids than are included in the drawing; in some cells they were tightly packed like a stack of coins. In side views of the plastids the starch grains appear as small ellipsoidal bodies, while in face views they have a spherical shape.

Kiesselbach (1916) found a marked difference in appearance between fixed and living bundle sheath plastids of corn. He believed that the fixing

fluid caused the plastids to assume an unusual crescent-like orientation in the cell. Our own observations, however, indicate that except for changes in position due to alterations in the size and shape of the plastids, it is doubtful if any movement of these plastids occurs upon their being fixed. When free-hand sections of fresh leaves were mounted in water, the plastids absorbed water and became distended. Fixation images of these plastids were very similar when fixed in Regaud's, Champy's, or Craf; their fixed appearance resembled that of living material mounted in paraffine oil.

In fixed material stained with haematoxylin the plastid of the bundle sheath appears as a flattened ellipsoid with a deeper-staining rim or shell partially enclosing a lighter-staining central core (figs. 1, 3, 6, 8). When viewed from different positions, these plastids present varying shapes owing to their asymmetrical form. Bundle sheath plastids, especially those which lack starch grains, in face views have a striking resemblance to the dictyosomes of the Golgi apparatus. The peripheral, more deeply staining rim is seen to contain in starch-free plastids numerous small colorless areas which appear like vacuoles. It is in these vacuolar-like regions of the plastid that starch is later deposited. The "vacuoles" and the starch grains have the same shape and approximate size. When starch is present, the grains are found embedded in but protruding from the surface of the plastid. The central region of the plastid contains neither starch vacuoles nor starch grains. The number of starch-forming "vacuoles" in a starch-free plastid may be equal to the number of starch grains subsequently formed by that plastid. It is possible, however, that as soon as one of these "vacuoles" has formed one mature starch grain, it begins the elaboration of a second, and so forth, so that the number of starch grains formed may be greater than the number of starch-forming regions. A decisive answer to this problem could be had either by an accurate determination of the number of "vacuoles" in a living plastid and the number of starch grains found later or by a statistical study of fixed material. In a single plastid the starch grains, which are arranged in several layers when viewed on edge, range in number from fewer than 20 to more than 40. The individual starch grains are easily detached from the surface of the plastid and may be found floating free in the mounting fluid. These detached grains are especially noticeable in iodine-stained preparations, where they appear as blue-colored ellipsoidal bodies. Although a plastid contains a number of starch grains, each grain is simple rather than compound. Figure 2 is an optical section of a young plastid containing 20 individual starch grains. Ten of the grains lie in one focal plane, the remainder lying above and below. These definite regions, the vacuoles, of the parenchyma sheath plastid which are concerned with starch formation are similar in function, though not in structure, to the pyrenoids of algal and moss plastids.

A comparable situation has been reported by Zirkle (1926) for the ordinary (mesophyll) plastid of *Elodea canadensis* which has a central vacuole containing a single starch grain. This vacuole is visible when no starch is present.

The parenchyma sheath cells enclosing young vascular bundles of maize have smaller plastids than do older cells; they not only have fewer starch grains but are less flattened than plastids of mature bundles.

The above description of the parenchyma sheath plastid differs in several fundamental respects from Zirkle's (1929) account. What he believes to be pores of the plastid leading to the centrally located vacuole are actually starch grains, which do not stain with haematoxylin and appear colorless

FIG. 2. Median optical section of a small parenchyma sheath plastid in side view showing arrangement of starch grains. These starch grains are embedded in the plastid but are separated from each other by thin layers of plastid substance. This plastid contains 20 starch grains. Ten are in one focal plane, the remainder lying above and below. The central region of the plastid is not visible when viewed from this position. This plastid is a much less flattened ellipsoid than those shown in figure 1.

(see Zirkle's figure 1). He believes that each plastid contains a single centrally located starch grain; but we have shown that these plastids have as high as 40 starch grains. Zirkle reported that the mesophyll plastids contained starch in their central vacuoles when conditions were especially favorable for photosynthesis. In our experiments with field-grown maize we found no trace of starch in these plastids either with the iodine test or with polarized light. This was true even though abundant starch was present in the bundle sheath plastids. We conclude that starch synthesis normally does not occur in the mesophyll plastids; they are devoted to the elaboration of soluble carbohydrates. That the differences in structure and function between the mesophyll and sheath plastids may be only a reflection of local

physiological conditions, especially the concentration of soluble sugars, and are not due to inherent differences is indicated by the observation of the remarkable transformation of the mesophyll plastids into sheath-like plastids by greatly increasing the amount of sugar present in the leaf. The cut ends of young maize leaves were immersed in a strong sucrose solution (27 grams of sucrose in 100 cc. of water). After 30 hours in the sugar solution the mesophyll plastids in the portion of the leaf just above the level of the solution each contained a number of simple starch grains. Seventy-eight hours after being placed in the sugar solution the change of the mesophyll plastids into sheath-like plastids was virtually complete. Except that the smaller mesophyll plastids contained fewer but larger starch grains they closely resembled the sheath plastids. In normally growing leaves the sugars manufactured in the mesophyll cells are moved into the bundle sheath cells. Presumably the sugar concentration of the mesophyll cells remains at a relatively low level while that of the bundle sheath becomes increasingly greater. Under such conditions the mesophyll plastids are concerned only with photosynthetic activity while the bundle sheath plastids elaborate, and temporarily store, starch. However if the sugar concentration of the mesophyll cells becomes unusually high it might be expected, on the basis of the above experiment, that the mesophyll plastids would contain starch.

It has long been known that the plastids of the bundle sheath cells of the corn leaf are green, although of a lighter color than the plastids of the mesophyll. If the green pigment is chlorophyll, these plastids may be capable of photosynthesis. The starch deposited within them might be derived from sugars synthesized there and not from soluble carbohydrates moved in from the surrounding mesophyll cells. The heavy deposit of starch found in these plastids would seem to be greater than could be accounted for by their photosynthetic activity, but actually nothing is known of their ability in this respect. Fortunately we were able to show unequivocally that the starch found in these plastids was derived from soluble carbohydrates made in the mesophyll plastids and translocated to the parenchyma sheath cells where the starch synthesis occurred. Certain of the maize leaves collected for study were green-and-white-striped. The plastids in the white regions were quite distinct from the normal plastids in the green area of the leaf (Rhoades 1943). At the transition zone between the green and white regions of one leaf a vascular bundle was found enclosed by parenchyma sheath cells with normal plastids. Adjacent to the sheath cells on one side of the bundle were mesophyll cells with normal green plastids, while next to the remaining sheath cells were mesophyll cells with abnormal plastids which lack chlorophyll and are incapable of photosynthesis. The plastids of the sheath cells touching green mesophyll cells were filled with starch grains, while the normal plastids of bundle sheath cells adjacent to colorless meso-

phyll cells contained no detectable starch. Not answered by this experiment is the question whether or not any photosynthesis takes place in the green bundle sheath plastids. A certain amount of sugar could be manufactured, but if the concentration were low, it could be immediately moved into the vascular bundle.

It has been tacitly assumed that the pale green color of the bundle sheath plastids is that of chlorophyll. Zirkle (1926) noted the lighter color of the sheath plastids of *Elodea* and suggested that they contained as much chlorophyll as the smaller mesophyll plastids but that it was stretched into a thinner layer in the larger sheath plastids. The question might be left open as to whether or not the green pigment in the bundle sheath plastids is the same kind of chlorophyll as in the mesophyll plastids. It has been shown that there is wide variation in the chlorophyll pigments throughout the plant kingdom. For example, Spoehr *et al.* (1942) have shown that certain algae lack chlorophyll b while containing chlorofucine. The possibility should not be summarily dismissed that even within an individual plant there may be variation in the kind of chlorophyll or in the ratio of chlorophyll a to chlorophyll b in different cells. Sayre (1926) found that the guard cells of *Rumex patientia* contained a green pigment which he believed on the basis of microchemical tests was not true chlorophyll. Since the bundle sheath plastid of the corn leaf differs from the mesophyll plastid in function and structure, the nature of their green pigments might not be identical.

DIURNAL CYCLE OF STARCH FORMATION BY BUNDLE SHEATH PLASTIDS

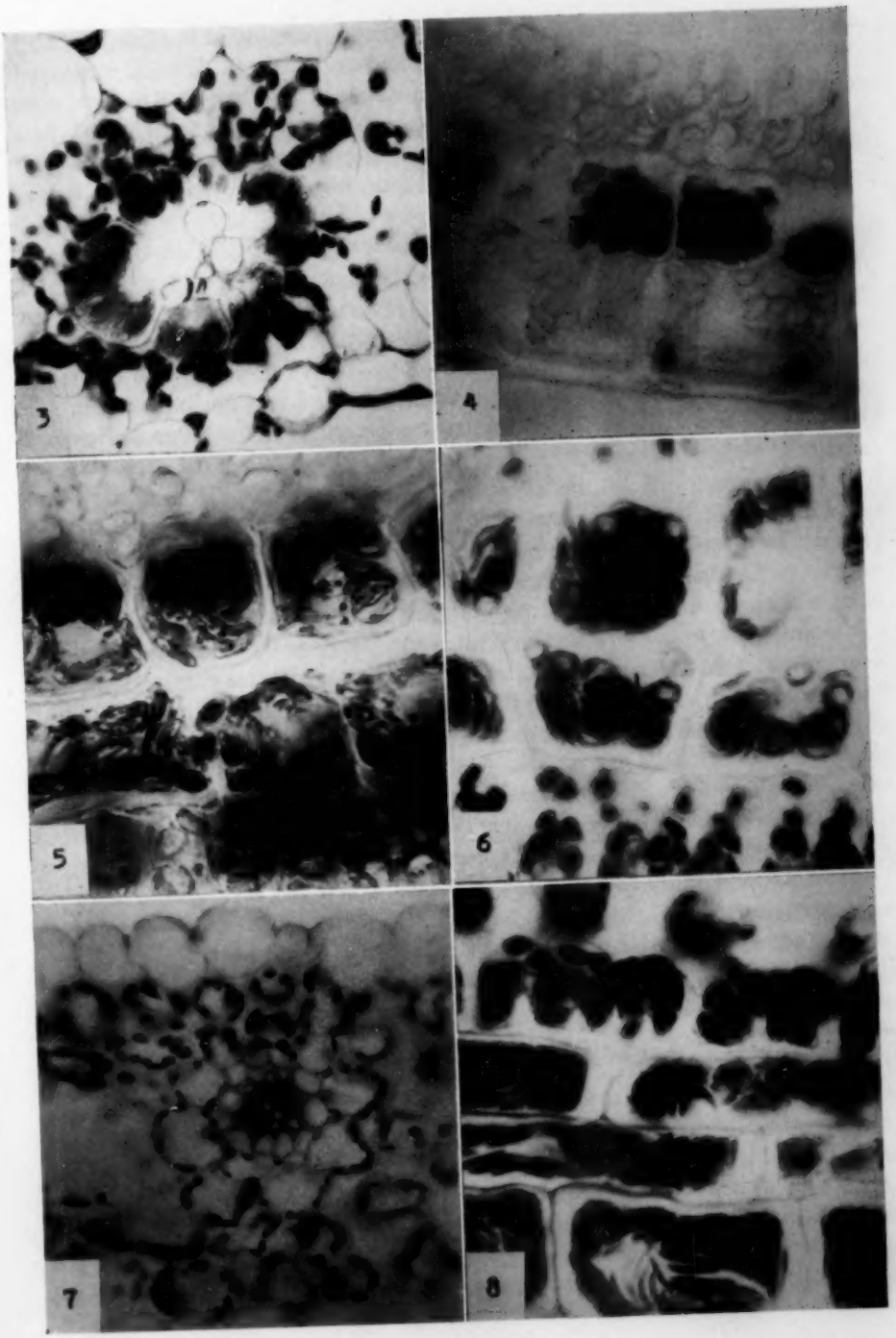
We have presented evidence that the starch deposited in the bundle sheath plastids comes from soluble carbohydrates made in the mesophyll cells and moved into the sheath cells where the transformation to starch occurred. In this section experiments are discussed which indicate that the transformation of soluble carbohydrates to starch occurs in the bundle sheath plastids only when the rate of movement of sugar into these sheath cells from the mesophyll cells is greater than the rate of translocation from the bundle sheath cells into the vascular bundles. Further, we shall show that the starch which increasingly accumulates throughout the day in these plastids is transformed back to soluble carbohydrates during the night, when no photosynthetic activity takes place, so that by morning the bundle sheath plastids are devoid of starch.

Leaf samples were taken approximately 6 inches from the tip of mature leaves from each of three plants at two-hour intervals beginning at 6:30 a.m. Eastern War Time on July 27, 1943, and ending at 12:30 a.m. on July 28. At 6:30 a.m. on July 28 another collection was taken from each of the three plants. Each sample consisted of a piece of leaf blade 2 cm. long and

1.5 cm. wide. Two samples only were taken from a single leaf; they came from opposite sides of the midrib and in all cases were collected at successive time intervals. Samples were taken only from the upper leaves of the main stalk or of the several tillers, where leaves were well exposed to the rays of the sun. Conditions were presumably excellent for photosynthesis, since the day was warm and there was an adequate supply of moisture in the soil. The leaf samples were fixed in Craf fluid. Some slides were stained with haematoxylin and some with a weak I–KI solution. Preliminary tests had shown that the bundle sheath plastids seemed devoid of starch when leaf samples were taken in the morning, while abundant starch was found later in the day. The leaf samples collected at 6:30 a.m., July 27, had bundle sheath plastids with no or little starch. In only an occasional sheath cell could starch be detected by I–KI. The 8:30 a.m. samples were completely devoid of starch; all starch present in these plastids had been hydrolyzed and translocated. Starch grains were found in the 10:30 a.m. samples but in a relatively slight amount as indicated by the intensity of their blue color with I–KI. The 12:30 p.m. samples had a markedly greater amount of starch. Plump starch grains protruded from the surface of the sheath plastids and gave an intense color with I–KI. All three of the 2:30 p.m. samples gave a weaker color with I–KI, indicating that less starch was present than at 12:30 p.m. This finding is important in view of the fact that the morning of July 27 was clear and bright. Beginning approximately at 1:00 p.m. and lasting until about 3:00 p.m., the sky became heavily overcast and the light intensity was greatly reduced. Presumably less photosynthetic activity occurred in the mesophyll cells during the overcast period; less sugar was passed into the bundle sheath cells than was moved into the vascular tissues,

Explanation of figures 3–8

FIG. 3. Cross section of maize leaf collected at 4:30 p.m., showing plastids of parenchyma sheath cells filled with starch grains. Fixed in Craf fluid; stained with haematoxylin. ×480. FIG. 4. Longitudinal section of maize leaf stained with weak I–KI. Starch grains in parenchyma sheath plastids deeply stained. Mesophyll plastids (above and below parenchyma sheath) give no indication of starch. Note starch in guard cells at lower right (slightly out of focus). ×480. FIG. 5. Longitudinal section of maize leaf stained with I–KI followed by partial removal of iodine with higher alcohols. The starch grains are clearly visible in the somewhat swollen plastids. ×480. FIG. 6. Longitudinal section of maize leaf collected at 6:30 a.m. Fixed with Craf fluid and stained with haematoxylin. Parenchyma sheath plastids are devoid of starch. Mesophyll cells lie to right. The deeper-staining rim or shell which partially encloses the lighter-staining central core is clearly visible in certain of the plastids. The vacuole-like regions of the deeper-staining rim where starch grains will be formed can be seen as light-staining regions. ×480. FIG. 7. Cross section of barley leaf showing parenchyma sheath cells with small plastids concentrated to one side of cell. These plastids take a deeper stain with haematoxylin than do the larger plastids of the mesophyll cells. ×360. FIG. 8. Longitudinal section of maize leaf collected at 6:30 a.m. Stained with haematoxylin. When seen in face view the sheath plastids resemble the dictyosomes of the Golgi apparatus. Note vacuole-like regions in deeper-staining rim of plastid. ×480.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so part of the starch accumulated by the sheath plastids in the late morning hours was hydrolyzed to soluble sugars and translocated. The skies cleared about 3:00 p.m. and the rest of the day was sunny and bright. The 4:30 p.m. samples contained a great quantity of starch, as did the 6:30, 8:30, and 10:30 p.m. samples. It is our impression that the greatest quantity of starch was present in the 6:30 and 8:30 p.m. samples, but no quantitative determinations were made.

The sun set at 8:18 p.m. on July 27. There was still a considerable amount of starch in the bundle sheath plastids of the samples collected at 12:30 the following morning but less than at 10:30 the night before. It was possible to find a few plastids with a faint blue color in the 6:30 a.m., July 28, samples, but the majority of the bundle sheath plastids gave no indication of starch with I–KI. All or nearly all of the starch formed in these plastids in the daylight hours of July 27 had been transformed back to sugar during the night.

The sun rose at 5:46 a.m. on July 27. It was a warm and sunny morning. A minor but detectable quantity of starch could be found in a few of the bundle sheath plastids of the 6:30 a.m. samples, while no starch was present in the 8:30 a.m. samples. This suggests that hydrolysis of the stored starch had not been quite completed by 6:30 a.m. but was finished by 8:30 a.m. It is not unreasonable to assume that some photosynthetic activity had occurred prior to 8:30 a.m. Presumably the photosynthetic rate at this time was so low that the rate with which sugar moved into the sheath cells was less than the rate of its passing from the sheath cells into the vascular elements; consequently no starch was deposited and the hydrolysis and translocation of the starch formed the previous day was completed. In the later hours of the day more sugar is manufactured than can be translocated by the vascular bundles, and this excess of sugar is temporarily stored as starch in the bundle sheath plastids. Later, when the cessation or slowing of photosynthesis occurs, this starch is hydrolyzed and translocated into the bundles. This interpretation is consistent with all observed facts and offers a logical explanation for the presence of the specialized plastids of the bundle sheath.

OBSERVATIONS ON OTHER GRASSES

Maize belongs to the subfamily Panicoideae which includes the Andropogoneae, the Maydeae, and the Paniceae. Schwendener (1890) found that the majority of the Panicoideae have a parenchyma sheath but no mestome sheath enclosing the vascular bundles. Sorghum is a member of the Andropogoneae. A study of leaf sections disclosed a situation similar to that in maize. The parenchyma sheath cells have unusually large, pale-green chloroplasts containing many starch grains. The occurrence of a diurnal cycle of starch

deposition and removal probably exists in these plastids as in maize, but this was not established.

The common cereals—oats, wheat, and barley—belong to the subfamily Pooideae. A cursory examination was made of their leaf histology. Our studies were made only on fixed sections of young leaves collected in the afternoon. Possibly different conditions would be found in older leaves, since Percival (1921) states that the outer sheath of older leaves of wheat contains few or no plastids. In these cereals each leaf bundle is surrounded by an inner and outer layer of cells. The parenchyma sheath cells have numerous plastids which, however, are smaller than the plastids of the mesophyll. The mestome sheath has no plastids. Only small amounts of starch, apparently lying in a central region of the plastid, were found in the parenchyma sheath plastids and also in the mesophyll plastids. The parenchyma and mesophyll plastids of wheat, oats, and barley had so little starch that their leaves gave only a perceptible color with I–KI. On the other hand, the great amount of starch found in the parenchyma sheath plastids of corn and sorghum gives their leaves an intense black color with I–KI. The parenchyma sheath plastids were found concentrated at the side of the cell bordering the mesophyll. The above observations hold for all three genera, but slight differences were noted. The greatest size difference between the sheath plastids and the mesophyll plastids was found in the barley leaf, although a difference in size exists in both wheat and oats (fig. 7). In structure and function, the parenchyma sheath plastids of wheat, oats, and barley, all members of the Pooideae, are obviously different from the bundle sheath plastids of maize and sorghum which belong to the Panicoideae. It will be of interest to ascertain to what extent the members of the two subfamilies of grasses differ in this respect.

SUMMARY

The parenchyma sheath cells enclosing the vascular bundles of the corn leaf contain specialized plastids, green in color, concerned with the elaboration of starch. The starch grains are formed in definite vacuole-like regions in the plastid. A plastid may have as many as 40 starch grains. Starch is deposited in these plastids only when the rate of movement of sugar from the mesophyll cells into the bundle sheath cells is greater than the rate with which sugar passes from the sheath cells into the vascular elements. The bundle sheath plastids deposit starch increasingly during the day. At night this starch is hydrolyzed to soluble carbohydrates and translocated so that the plastids are free of starch by morning. No starch was found in the mesophyll plastids at any time during the day or night. The bundle sheath plastids of sorghum are similar in function to those of maize; this specialization of the bundle sheath plastids may be typical of the subfamily

Panicoideae. The parenchyma sheath plastids of wheat, oats, and barley, all members of the subfamily Pooideae, are different in size and function from those of maize and sorghum.

DEPARTMENT OF BOTANY, COLUMBIA UNIVERSITY
NEW YORK

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THE INHERITANCE OF CERTAIN CHARACTERS IN A CROSS OF TWO AMERICAN SPECIES OF LACTUCA

THOMAS W. WHITAKER

INTRODUCTION

Two American species of *Lactuca*, *L. canadensis* L. and *L. graminifolia* Michx., are endemic in the eastern portions of the United States and Canada. In general *L. canadensis* ranges from Nova Scotia westward to Saskatchewan and as far south as Georgia, Alabama, Louisiana, and Arkansas and occurs in dry or moist open ground. *Lactuca graminifolia* is found farther south than *L. canadensis*. Its range extends from South Carolina to Florida and west as far as Texas. It usually occurs in rich soil in fields or woods.

Lactuca canadensis and *L. graminifolia* have seventeen pairs of chromosomes (Babcock *et al.* 1937). There is good evidence that the American species of *Lactuca* (with one exception) are amphidiploids, although the exact steps in their origin remain obscure. They are assumed to have originated by hybridization of Old World species of *Lactuca* with eight and nine chromosomes followed by amphidiploidy.

The present study was initiated with the primary purpose of investigating all available species of *Lactuca* as possible sources of desirable genes for introduction into cultivated lettuce, *L. sativa*. The data reported in the present paper have been incidental to our main project, but it is felt that these observations may contribute to a better understanding of the characters which differentiate certain species in nature. In analyzing the relations between these two species (*L. canadensis* and *L. graminifolia*) it may be possible to throw some light on the hypothesized origin of the American species of *Lactuca*.

The relationships between these two species are unique, and differ in detail from other reported cases of this kind (see Goodwin 1938; Winge 1938). First, they are quite distinct entities morphologically, and would be considered "good species" by even the most conservative taxonomist. Second, in reciprocal crosses the F_1 and succeeding generations are extremely fertile. Third, the species under consideration are not ordinary diploids or tetraploids, but euploids of some sort, most probably amphidiploids. Fourth, both species are predominantly self-pollinated and are self-fertile.

As mentioned above, both *Lactuca graminifolia* and *L. canadensis* have seventeen pairs of chromosomes. Usually there is little or no multivalent formation, the entire complement being composed of bivalents at first metaphase. In the F_1 hybrid there is regular pairing of the chromosomes of the

two parental species. An examination of several F_2 plants indicates that meiosis follows a normal course in all of those examined.

DESCRIPTION OF MATERIALS

In 1933 the late J. B. Norton observed in the vicinity of Hartsville, South Carolina, plants which seemed to be intermediate between *Lactuca canadensis* and *L. graminifolia*. These plants were found in locations where the two species were growing together, and were evidently segregates, produced as a result of natural crossing between them.

Norton collected seed of both species, and of the intermediate plants. Starting with seed from this source, the present study has extended over a period of several years. We were able to produce, without difficulty, F_1 hybrids between the two species. The F_1 and plants from subsequent generations proved to be fully as fertile as the parental species. Under our conditions both species have been very uniform. The two species and the F_1 are described below (see figures 1, 2).

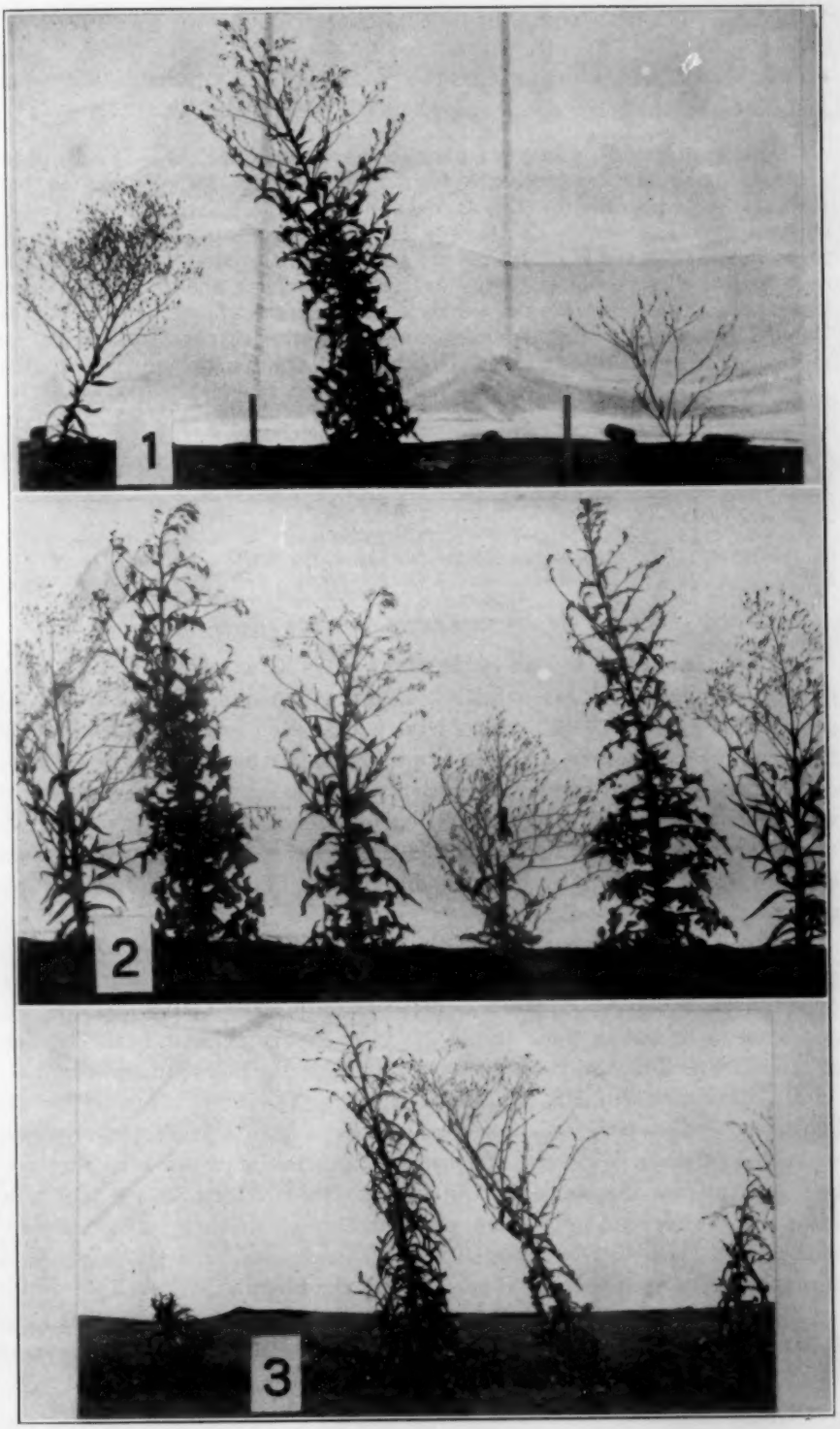
Description of Lactuca canadensis L. Stems stout, erect, 2–3 m. high, leafy, glabrous, paniculate above; basal leaves sparingly hirsute with scattered long and short hairs on upper surface, and long hairs on midrib both above and below; cauline leaves largely glabrous with few long hairs on midribs; basal leaves spatulate, dentate to pinnatifid; cauline leaves sinuate pinnatifid; involucres 10–12 mm. long; bracts linear-lanceolate to lanceolate; pollen grains yellow; ligules orange yellow; achenes flat, oval, black, narrow-margined, with single prominent rib in the middle of each face, 3–4 mm. long, contracted into slender beaks 1–2 mm. long; biennial, seed planted in the spring makes only rosette of leaves until the following spring when seed stalk develops.

Description of Lactuca graminifolia Michx. Stems slender, 0.5–1.0 m. high, glabrous, paniculate above; leaves linear, basal mostly pinnatifid, with a few narrow spreading or deflexed lobes, others entire; involucres 18–20 mm. long; bracts linear-lanceolate to lanceolate; pollen grains grey; ligules purple-blue, achenes flat, oblong-elliptical, dark purple-brown, narrow-margined, with single prominent rib in the middle of each face, 5–6 mm. long, contracted into slender beaks 3–4 mm. long; in South Carolina, according to Norton, normally a winter annual blooming early in the spring from autumn sown seed; in coastal Southern California it grows well as either a winter or a summer annual.

Reciprocal cross pollinations between *Lactuca canadensis* and *L. graminifolia* during three seasons have invariably produced an abundance of viable

Explanation of figures 1–3

FIG. 1. Left to right: F_1, *Lactuca canadensis*, *L. graminifolia*. FIG. 2. F_2 segregates from the cross *Lactuca canadensis* × *L. graminifolia*. FIG. 3. F_2 individuals showing segregation for growth habit. Annuals for the most part in flower. Low plants in foreground are biennials; no indication of seed stalk formation.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3

crossed seed. Each species appears to produce seed fully as abundantly when pollinated by the other species as when self-pollinated.

Description of F_1 (Lactuca canadensis × graminifolia). The F_1 plants are approximately intermediate (fig.1) between the two species in most respects, including height, size and shape of rosette leaves, color and shape of ligules, color and size of achenes. In our work the annual habit proved to be dominant over biennial habit; grey color of pollen grains dominant over yellow color. Lobing of the cauline leaves was dominant over entire leaves, although they were not nearly as pinnatifid as *L. canadensis* (fig. 4). Table 1 lists the prominent contrasting characters of the two species, and indicates the appearance of these characters in the F_1.

TABLE 1. *Table of prominent contrasting characters.*

	L. canadensis	*L. graminifolia*	F_1
Growth habit	biennial	annual	annual
Ligules	orange yellow	purple blue	purple
Pollen	orange	grey	grey
Involucral bracts (length)	13–14 mm.	19–20 mm.	18–19 mm.
Cauline leaves	lobed, pinnatifid	entire, lanceolate	lobed
Height	2–3 m.	0.5–1.0 m.	1.2–2.5 m.

EXPERIMENTAL RESULTS

A. **Inheritance of Pollen Grain Color.** As noted above, *Lactuca canadensis* produces pollen grains with a deep orange color; *L. graminifolia* has grey pollen grains. The F_1 plants have grey pollen grains, indicating that the gene for grey color of pollen grains is dominant over that for orange color. This gene segregates very sharply in the F_2 with no suggestion of intermediate colors. In this case pollen color acts as an ordinary sporophytic character, since there is no segregation for color in the F_1 plants. It seems likely that the substances responsible for color are produced by the sporophyte.

From F_1 plants grown in 1937 three F_2 families were obtained. A total of 635 individuals were scored for pollen color in the F_2; the results are recorded in table 2. Of ten F_3 families, four were segregated and six appeared to be homozygous (table 3). It is clearly evident from the data of tables 2 and 3 that a single gene is responsible for pollen grain color.

B. **Inheritance of Plant Growth Habit.** The inheritance of growth habit has been investigated in several species of plants belonging to widely separated families. With one exception the annual habit has been shown to be dominant over the biennial habit (Clarke 1935; Abegg 1936), and in all cases it was governed by a single gene difference. Melchers (1937) reports that growth habit in *Hyoscyamus niger* is controlled by a single gene; in this species the annual habit is recessive to the biennial.

As noted in a previous report (Thompson *et al.* 1941), the annual habit is dominant over the biennial in several interspecific crosses of *Lactuca*. A

TABLE 2. *Segregation for pollen color in F_2 families.*

Family No.	No. of plants	Pollen color				χ^2
		Orange	Grey	Expected orange	Expected grey	
13454	554	137	417	138.5	415.5	0.04
33111–1	38	9	29	9.5	28.5	0.017
33112–1	43	16	27	10.7	32.1	3.42
Total	635	162	473	(df = 3, P = 0.4)		3.477

limited number of observations have been made on the inheritance of annual vs. perennial growth habit. In nature *Lactuca graminifolia* is an annual and *L. canadensis* is biennial. To determine the quantitative expression of this character, seed of the parental species, the F_1, and one family of F_2

TABLE 3. *Segregation for pollen color in F_2 families.*

Family No.	Phenotype of F_1 parent	No. of plants	Pollen color		χ^2
			Orange	Grey	
30001	grey	10	....	10	
30003	orange	19	19	....	
30004	grey	17	....	17	
30005	grey	5	1	4	0.06
30008	grey	24	5	19	0.22
30009	orange	15	15	....	
30010	orange	10	10	....	
30011	grey	72	24	48	1.18
30012	grey	70	47	23	2.30
30013	orange	3	3	....	

plants were planted during the early part of August. After four weeks in the seed bed the plants were transplanted to the field and were scored for growth habit from October through November. At this time (11/10/39) our records show that all plants of *L. graminifolia* and the F_1 hybrids were in full flower. Plants of *L. canadensis* did not produce seed stalks until the

TABLE 4. *Segregation of annual vs. biennial habit of growth in F_2 and F_3 families.*

Family No.	Phenotype of parent	Total No. of plants	Annual	Biennial	χ^2
13454 (F_2)	annual	334	257	77	0.67
33111–1–16 (F_3)	"	7	6	1	
33111–1–18 (F_3)	"	16	12	4	
30004 (F_2)	biennial	23	0	23	
30005 (F_2)	annual	10	4	6	
33111–1–33 (F_3)	"	10	10	....	
30008 (F_2)	"	17	10	7	
33112–1–33 (F_3)	biennial	10	0	10	

following March. Table 4 shows the segregation in the F_2 family and a few of the F_3 observations (fig. 3). These rather meager observations, while not conclusive, seem to indicate that growth habit is probably dependent upon a single gene difference.

C. **Inheritance of Leaf Lobing.** The basal or rosette leaves of *Lactuca graminifolia* are linear and pinnatifid, usually with several narrow lobes either spreading or deflexed; in contrast the cauline leaves are linear-lanceolate and entire. On *L. canadensis* the rosette and flower-stalk leaves are of the same general character, spatulate and dentate to sinuate pinnatifid. In the F_1, leaf shape is approximately intermediate between the two parental species, but both rosette and cauline leaves are distinctly pinnatifid (fig. 4). In the F_2, most plants have rosette leaves which are more or less pinnatifid. In some cases only one leaf of the rosette will be lobed. With regard to the cauline leaves there is segregation into two types, lobed or

TABLE 5. *Segregation for lobing in F_2.*

Family No.	Phenotype of parent	Total No. of plants	Lobed	Entire	χ^2
13454	lobed	746	582	164	3.62

pinnatifid and entire (fig. 5). Records from one F_2 family of 746 individuals are summarized in table 5. Of ten F_3 families, four appeared to be homozygous and six were segregating. However, the number of plants scored from each family was not sufficient to make this determination accurate, but there is strong evidence from the one F_2 family of 746 individuals that the basic leaf pattern is controlled by a single gene.

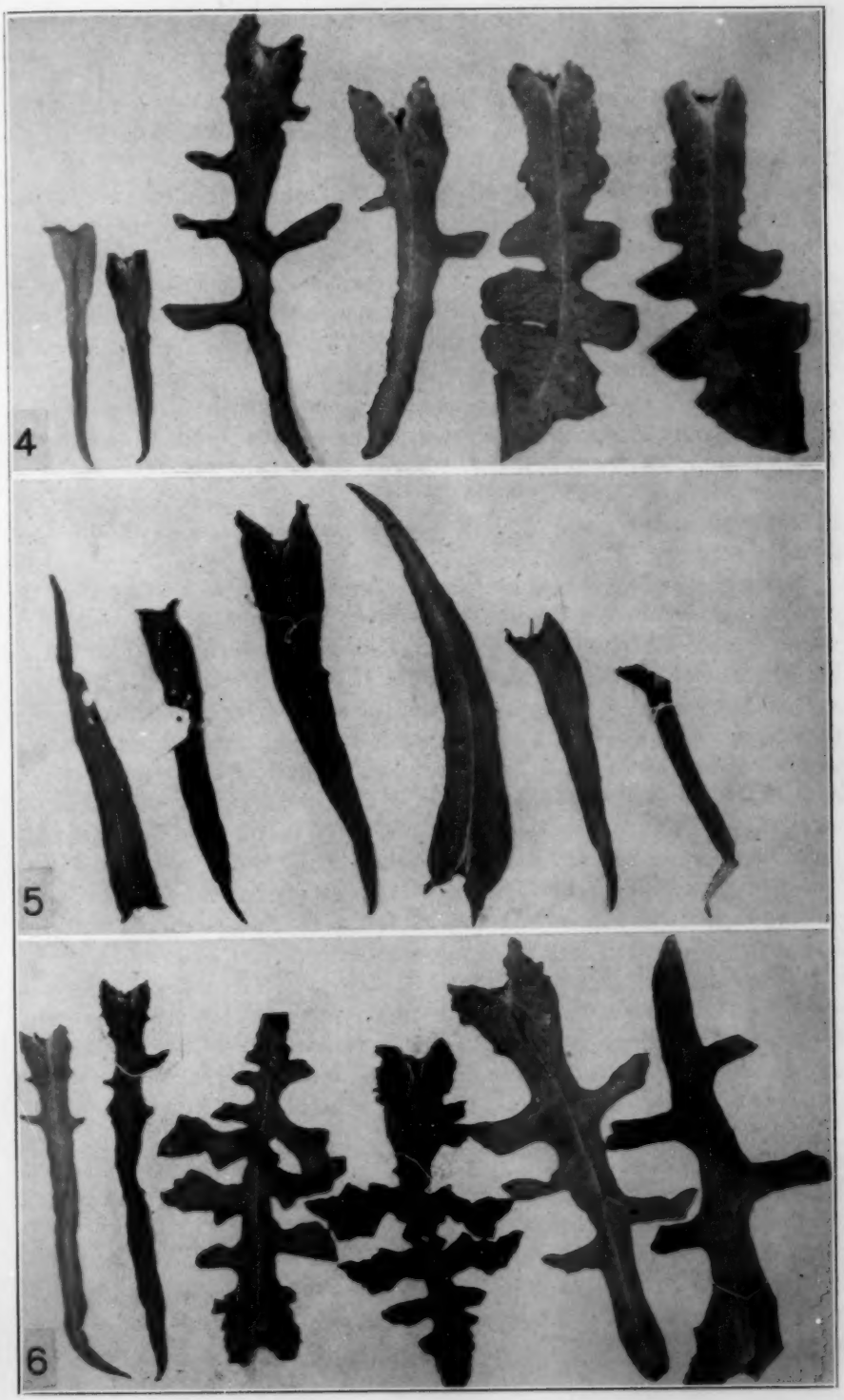
Although the basic pattern of the leaf (lobed vs. entire) seems to be governed by a single gene, there is apparently a group of modifiers at work which are responsible for the number and shape of the lobes. No attempt has been made to analyze these factors.

DISCUSSION

The characters selected for study all are apparently simple Mendelian gene differences. However, there are a number of other segregating char-

Explanation of figures 4–6

FIG. 4. Cauline leaves—two leaves from each plant, dorsal and surface view; left to right: *Lactuca graminifolia*, entire; F_1 (*L. graminifolia* × *L. canadensis*), lobed; *L. canadensis*, lobed. FIG. 5. Cauline leaves—entire leaves from three F_2 segregates (dorsal and surface view of leaves from each plant). FIG. 6. Cauline leaves—lobed leaves from three F_2 segregates (dorsal and surface view of leaves from each plant). Note the variation in the pattern of lobing, from the minimum condition on the left to the highly pinnatifid condition of the center leaves.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acters which appear to have a more complex basis. With respect to ligule color, F_2 individuals range from those with a light orange color quite similar to *Lactuca canadensis* to individuals almost lacking in color or with only a faint tinge of purple.

If these two species are amphidiploids, the fact that bivalents are formed regularly at first meiotic metaphase indicates that they were derived from hybridization between rather distantly related species (Poole 1932; Huxley 1943). On this basis segregation for at least some of the character differences between the two amphidiploids should follow along simple lines, and this has been supported by actual observations. This evidence when combined with the work of Thompson (1942), who has actually synthesized an amphidiploid *Lactuca* by crossing a seventeen-chromosome species with a nine-chromosome one, lends indirect support to the original postulate of Babcock, Stebbins and Jenkins (1937) that the seventeen-chromosome species of *Lactuca* are amphidiploids obtained from a cross (or several crosses) between a nine-chromosome and an eight-chromosome species followed by doubling.

From the evidence produced in this investigation it is clear that the conspicuous differences between these two species are for the most part simple gene differences, probably on the same level as those which distinguish many varieties of cultivated plants. It is also probable that these two species are very closely related, having diverged from a common amphidiploid ancestor. The fact that the species are able to maintain themselves as distinct entities in nature is certainly not related to the lack of fertility of the hybrids, since all of the individuals resulting from the cross are fully as fertile as either species. It seems more likely that lack of success of the hybrids in nature can be attributed to their inability to encounter an ecological niche to which they are well adapted. One further fact seems important in this connection. I am told by Dr. R. C. Thompson that in nature *L. graminifolia* flowers well in advance of *L. canadensis*, and ordinarily has completed the flowering cycle before the flowers of *L. canadensis* begin to open. This differential timing of the reproductive cycle probably accounts for the fact that there is very little hybridization of these two species even though there is a decided overlapping of their respective ranges.

SUMMARY

1. Two species of *Lactuca*, *L. graminifolia* and *L. canadensis*, have been successfully hybridized. The F_1 progeny and plants of subsequent generations are as fertile as either parent.

2. A genetic analysis of three marked differences between these two species has shown that pollen color (grey vs. orange), leaf lobing (lobed vs. entire), and growth habit (annual vs. biennial) are each dependent upon single-gene differences.

3. The evidence accumulated in this work seems to indicate that the differences between these two very distinct species are for the most part dependent upon simple gene differences.

4. The relation of the observations reported in this paper to the theory of the amphidiploid origin of the seventeen-chromosome *Lactucas* is discussed.

U. S. Horticultural Field Station
La Jolla, California

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CYTOGENETIC STUDIES OF HYBRIDS WITH "MAKHA" WHEAT

T. C. CHIN AND C. S. CHWANG[1]

INTRODUCTION

On the basis of their chromosome numbers, the wheats fall into three groups: the so-called diploid wheats with $n = 7$, the tetraploids with $n = 14$, and the hexaploids with $n = 21$. These groups are taxonomically, as well as cytologically, distinct. Within each group the various species, with the notable exception of *Triticum timopheevi* of the tetraploid group, have homologous chromosomes which pair normally in hybrids. Crosses between groups show that the 21 different chromosomes of the hexaploids are composed of three sets (genoms) of seven, which are commonly designated A, B, and C, respectively, while the chromosomes of the tetraploids consist of sets A and B, and those of the diploids of set A. *Triticum timopheevi* is designated AAGG by Lilienfeld and Kihara (1934) and Kihara (1937), and AA$\beta\beta$ by Kostoff (1936).

Although the designation of the various sets of chromosomes by letter is a convenient shorthand device, it does not give an entirely accurate picture, particularly as regards the A set. As Kihara (1937) has pointed out, the A genom of the diploid wheats is not truly homologous with the A of the tetraploids, although the two sets are capable of forming up to seven pairs in hybrids. Even in hexaploid × tetraploid hybrids, where the respective A and B sets ordinarily show normal pairing, multivalent configurations and other irregularities are found when certain varieties are used (Love 1941; Chin & Chwang 1942). That there are intergenomic and possibly intragenomic homologies is shown by the fact that one or more pairs may be formed in haploids of wheats of the hexaploid and tetraploid groups. This conclusion is borne out by the occurrence of trivalents in tetraploid × diploid hybrids (Kihara & Nishiyama 1928; Aase 1930) and of various multivalents in hexaploid × tetraploid hybrids. These multiple configurations are presumably due to autosyndesis conditioned by structural changes between nonhomologous chromosomes (Darlington 1937; Love 1941; Chin 1942; Chin and Chwang 1942).

[1] This paper was transmitted to the editor by Dr. Barbara McClintock, to whom it was sent by T. C. Chin. In preparing it for publication, the editor had the generous cooperation of Dr. E. R. Sears. Because of current conditions, it was necessary to dispense with the usual intercourse between authors, reviewer, and editor; the authors could not be consulted about some of the modifications made by Dr. Sears, nor had they an opportunity of seeing the paper in proof. To publish the numerous tables recourse was had to the Lucien M. Underwood Memorial Fund.

Triticum macha Dekapr. & Menab. is a new species of wheat of the hexaploid or *vulgare* group found in West Georgia (Dekaprelevitch & Menabde 1932). Its discoverers regarded it as a possible ancestor of the present-day *T. spelta*, which it resembles in the toughness of its glumes. It is distinct from *T. spelta*, however, in the method of disarticulation of the rachis (Hector 1936), *T. spelta* fracturing below the point of attachment of the spikelets and *T. macha* above. As far as the present authors are aware, cytogenetic evidence of its relationship with other wheats is as yet lacking. This paper is a preliminary report of a series of cytogenetic investigations of hybrids between "Makha" and other wheats, with special emphasis on possible phylogenetic relationships.

MATERIAL AND METHODS

Triticum macha was obtained from the Kansas State Agricultural College, Manhattan, Kansas. It was crossed with the two hexaploid species *T. vulgare* and *T. spelta*, and with the three tetraploid species *T. durum*, *T. turgidum*, and *T. dicoccoides* in the summer of 1941. Both *T. vulgare* and *T. spelta* were beardless, while *T. turgidum*, *T. durum* var. *leucurum* Körn., and *T. dicoccoides* var. *Aaronsohni* were all fully bearded. *T. macha* has short awns. Spikes and spikelets of most of the species used are shown in figures 1 and 3.

The cytological studies were made from aceto-carmine smears prepared in the summer of 1942 at Chengtu. The majority of the slides were mounted in Zirkle's fluid, and the rest in Canada balsam. The drawings were made with the aid of a camera lucida.

GENETICAL OBSERVATIONS

Hexaploid Hybrids. 1. *T. vulgare* × *T. macha*. Only two hybrid plants were obtained—one from each of the two *vulgare* varieties used. Both plants showed signs of chlorophyll deficiency and were very weak and completely sterile. No pollen mother cells could be found. The spikes (fig. 2, no. 2; fig. 3, no. 7) were beardless, as in the *vulgare* parents, whereas the rachis was fragile, as in *T. macha*. Thus fragility of rachis and beardlessness were apparently completely dominant.

2. *T. spelta* × *T. macha*. The F_1 plants of this cross (fig. 2, no. 1; fig. 3, no. 6) were also very weak and showed signs of chlorophyll deficiency, but they reached maturity. The amount of sterility was 63.5 per cent. The seedlings were prostrate, as were those of both parents. The spike was of intermediate density and was beardless like *T. spelta*. The rachis was fragile both above and below the spikelets, showing that both types of disarticulation tend to be dominant, and suggesting that two independent genes are involved. The shoulder of the outer glume was narrow, as in *T. macha*.

Fig. 1. Spikes of (1) *T. spelta*, (2) *T. macha*, (3) *T. dicoccoides*, (4) *T. turgidum*, and (5) *T. durum*. ×0.2. Fig. 2. Spikes of (1) *T. spelta*×*T. macha*, (2) *T. vulgare*×*T. macha*, (3) *T. macha*×*T. dicoccoides*, (4) *T. macha*×*T. turgidum*, and (5) *T. macha*×*T. durum*. ×0.2. Fig. 3. Spikelets of (1) *T. spelta*, (2) *T. macha*, (3) *T. dicoccoides*, (4) *T. turgidum*, (5) *T. durum*, (6) *T. spelta*×*T. macha*, (7) *T. vulgare*×*T. macha*, (8) *T. macha*×*T. diccoccoides*, (9) *T. macha*×*T. turgidum*, and (10) *T. macha*×*T. durum*. ×0.2.

Pentaploid Hybrids. All three of these hybrids (fig. 2, nos. 3–5; fig. 3, nos. 8–10) had awns of intermediate length and spikes much longer than those of either parent. The degree of sterility was 37.4 per cent in *T. macha* × *T. turgidum*, 40.5 per cent in *T. macha* × *T. durum*, and nearly 100 per cent in *T. macha* × *T. dicoccoides*. Most of the F_1 plants of the last combination were completely sterile.

CYTOLOGICAL OBSERVATIONS

Hexaploid Hybrid. Associations of from one to six chromosomes were observed at first meiotic metaphase in *T. spelta* × *T. macha* (table 1). The

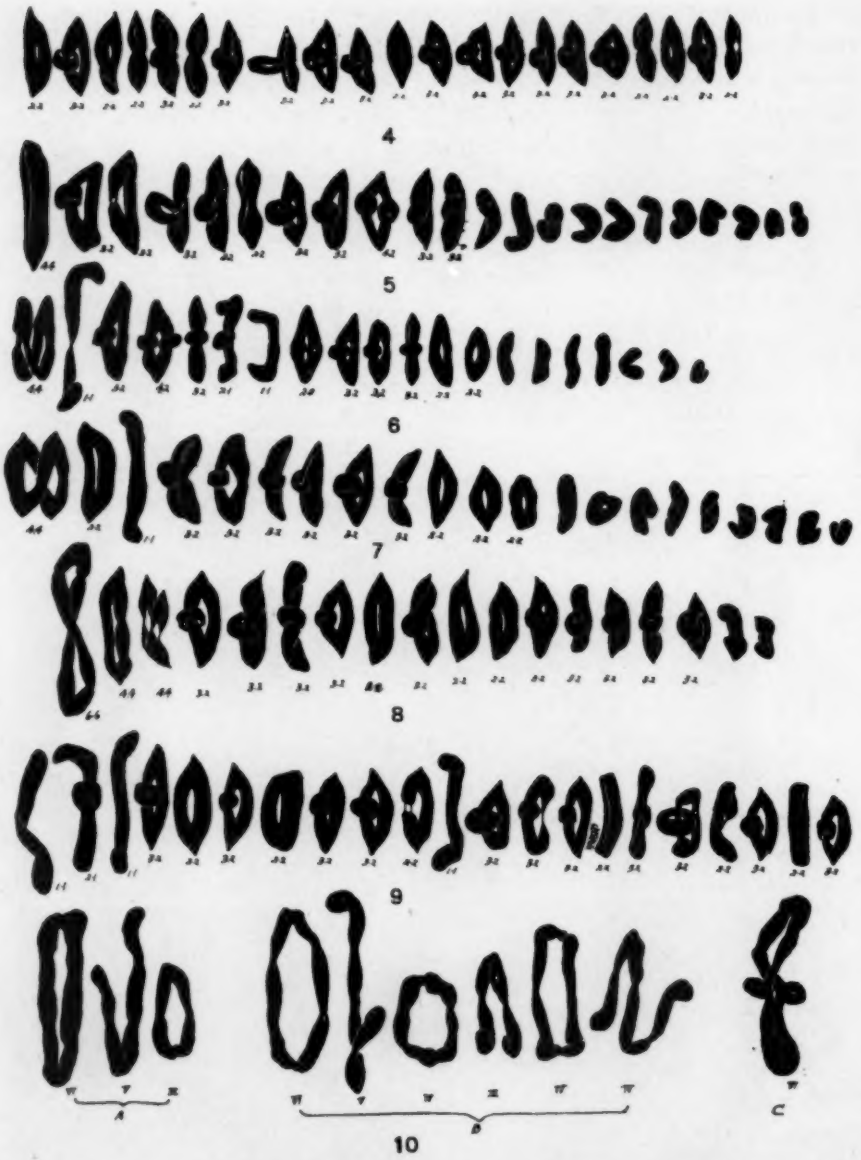
TABLE 1. *Combinations of configurations observed in Triticum spelta* × *T. macha.*

Number of microsporocytes	Configurations					
	I	II	III	IV	V	VI
1	2	13	....	2	....	1
1	2	15	....	1	....	1
2	2	17	....	....	....	1
1	1	18	....	....	1	....
1	3	17	....	....	1	....
1	5	16	....	....	1	....
1	2	16	1	....	1	....
1	....	15	....	3	....	....
3	....	17	....	2	....	....
1	2	16	....	2	....	....
2	1	17	1	1	....	....
2	....	18	2	....	....	....
3	2	17	2	....	....	....
5	....	19	....	1	....	....
6	2	18	....	1	....	....
7	1	19	1	....	....	....
1	3	18	1	....	....	....
29	....	21	....	....	....	....
3	2	20	....	....	....	....
3	4	19	....	....	....	....
74	0.93	19.08	0.28	0.36	0.05	0.05

average number of chromosomes involved in multiple configurations was 2.90, and the maximum number was 14—in one association of six and two associations of four (fig. 8). The maximum number of associations of four in the same cell was three. Of 74 cells, 39 (52.7 per cent) had multiple configurations; and 20 of these 39 had more than one multiple configuration. Twenty-nine of the 74 cells had 21 bivalents, as in figure 9.

The pairing intensity in the hybrid, as measured by the number of half-chiasmata per metaphase chromosome, was somewhat lower than in *T. macha* itself (fig. 4)—2.16 compared with 2.45. Even the bivalents considered by themselves had only 2.30 half-chiasmata per chromosome in the hybrid. Table 2 summarizes the data on the number of chiasmata in the various types of configurations.

FIG. 3. Spikelets of (1) *T. spelta*, (2) *T. macha*, (3) *T. dicoccoides*, (4) *T. turgidum*, (5) *T. durum*, (6) *T. spelta* × *T. macha*, (7) *T. vulgare* × *T. macha*, (8) *T. macha* × *T. dicoccoides*, (9) *T. macha* × *T. turgidum*, and (10) *T. macha* × *T. durum*. × 0.2.



FIGS. 4–9. First meiotic metaphase configurations in (4) *Triticum macha*, (8, 9) *T. spelta* × *T. macha*, (5) *T. macha* × *T. turgidum*, (6) *T. macha* × *T. durum*, and (7) *T. macha* × *T. dicoccoides*. Each figure shows the chromosomes from a single microsporocyte. The two numbers accompanying each bivalent and multivalent indicate the number of chiasmata (left) and the number of chromosomes (right) involved in that configuration. × 730. FIG. 10. Multivalent configurations in (A) *T. macha* × *T. turgidum*, (B) *T. macha* × *T. durum*, and (C) *T. spelta* × *T. macha*. × 730.

Terminal chiasmata constituted 82.8 per cent of the total in *T. spelta* × *T. macha*. The number of interstitial chiasmata was more variable from cell to cell than was the number of terminal chiasmata. This may be attributed to the tendency for terminal affinity to cause the localization of metaphase chiasmata in the terminal position (Darlington 1937). Also, a possible source of variation in the number of interstitial chiasmata lies in the structural changes of certain chromosomes, these changes presumably being capable of preventing the terminalization of certain chiasmata.

TABLE 2. *Frequencies of configurations with various numbers of chiasmata. Values are given in percentages of the total number of chromosomes observed of each hybrid.*

			6x	5x		
		Hybrids:	*T. spelta* × *T. macha*	*T. macha* × *T. turgidum*	*T. macha* × *T. durum*	*T. macha* × *T. dicoccoides*
Types of configuration and number of chiasmata	I	0	3.5	21.7	21.6	20.8
	II	1	9.2	4.3	8.0	4.2
		2	41.9	21.4	30.8	27.5
		3	33.2	40.6	30.6	33.8
		4	0.9	1.1	2.9	.....
	III	2	3.1	1.7	3.0	0.8
	IV	3	1.9	4.0	1.7	3.1
		4	3.2	2.9	0.6	8.3
		5	.....	0.6	.....	.....
	V	4	1.2	.....	.....	.....
	VI	6	1.4	.....	0.9	1.6
		7	.....	1.7	.....	.....
		8	0.5	.....	.....	.....
No. cells observed			30	20	20	11

A correlation of – 0.151 was found between the number of chiasmata per cell and the number of univalents. This value is statistically insignificant, suggesting that failure of pairing between one homologous pair of chromosomes may result in an increase in the number of chiasmata in some other pairs (Ribbands 1937).

The association of six was always in the form of a ring (figs. 8, 10 C), while the associations of five and of three were always chains.

Chromosome bridges were observed in 13.8 per cent of the microsporocytes at first anaphase (table 3). These bridges presumably resulted from crossing-over within inverted or duplicated segments, although few were accompanied by fragments. The bridges appeared to involve the long arm of chromosomes with subterminal centromeres (fig. 16).

No basis was found in the cytological data for the high degree of sterility (63.5 per cent) of this hybrid. Examination of pollen grains showed only 17.8 per cent aborted grains.

Pentaploid Hybrids. The pentaploid hybrids also had associations of from one to six chromosomes (table 4; figs. 5–7, 10 A, B). In the average number per cell of the various configurations, these hybrids were very similar to certain of those reported by Love (1941) between varieties of *T. vulgare* and varieties of *T. dicoccum* and *T. durum*—notably the crosses involving the *durum* variety Pentad. For *T. vulgare* var. McMurachy × *T. durum* var. Pentad, he found almost exactly the same average numbers of the various configurations as the combined average for the three hybrids reported here.

TABLE 3. *Frequency of bridges at first and second anaphase.*

Hybrid	Stage of meiosis	No. bridges observed	No. cells examined	Per cent with bridges
T. spelta × *T. macha*	Anaphase I	12	87	13.8
	Anaphase II	0	Many	0.0
T. macha × *T. turgidum*	Anaphase I	10	265	3.8
	Anaphase II	26	178	14.6
T. macha × *T. durum*	Anaphase I	4	240	1.7
	Anaphase II	42	274	15.4
T. macha × *T. dicoccoides*	Anaphase I	2	37	5.4
	Anaphase II	2	10	20.0

The fact that several microsporocytes had as few as four or five univalents suggests that there was some pairing among chromosomes of the C genom of *T. macha*.

Apparently the hexaploid hybrid involving *T. macha* had the capacity for forming one more association of four than had the pentaploid hybrids. This is indicated by the fact that one association of six and one of four, or two associations of four, were the maximum amount of multivalent formation in the pentaploids; whereas one association of six and two of four, as well as three associations of four, were found in the hexaploids. It is not clear whether the additional association of four in the hexaploid was composed entirely of chromosomes from the C genom, or whether it was composed partly of chromosomes from the C genom and partly of chromosomes from the A or B genom.

As in the hexaploid hybrid, the association of six was always in the form of a ring, and the associations of five and three were always chains.

The number of half-chiasmata per chromosome, when calculated on the basis of 14 possible pairs (that is, 28 chromosomes per cell), was 2.41 for *T. macha* × *T. turgidum*, 2.29 for *T. macha* × *T. durum*, and 2.33 for *T.*

macha × *T. dicoccoides*. These figures are to be compared with 2.45 for *T. macha* itself and 2.16 for *T. spelta* × *T. macha*. It thus appears that the pairing intensity is more nearly normal in the pentaploids than in the hexaploid hybrid. In fact, when calculations are made for only the bivalents actually present, the values become 2.57, 2.39, and 2.45, respectively, for the three pentaploid hybrids—approximately the same as for *T. macha* itself. As in

TABLE 4. *Combinations of configurations observed in pentaploid hybrids.*

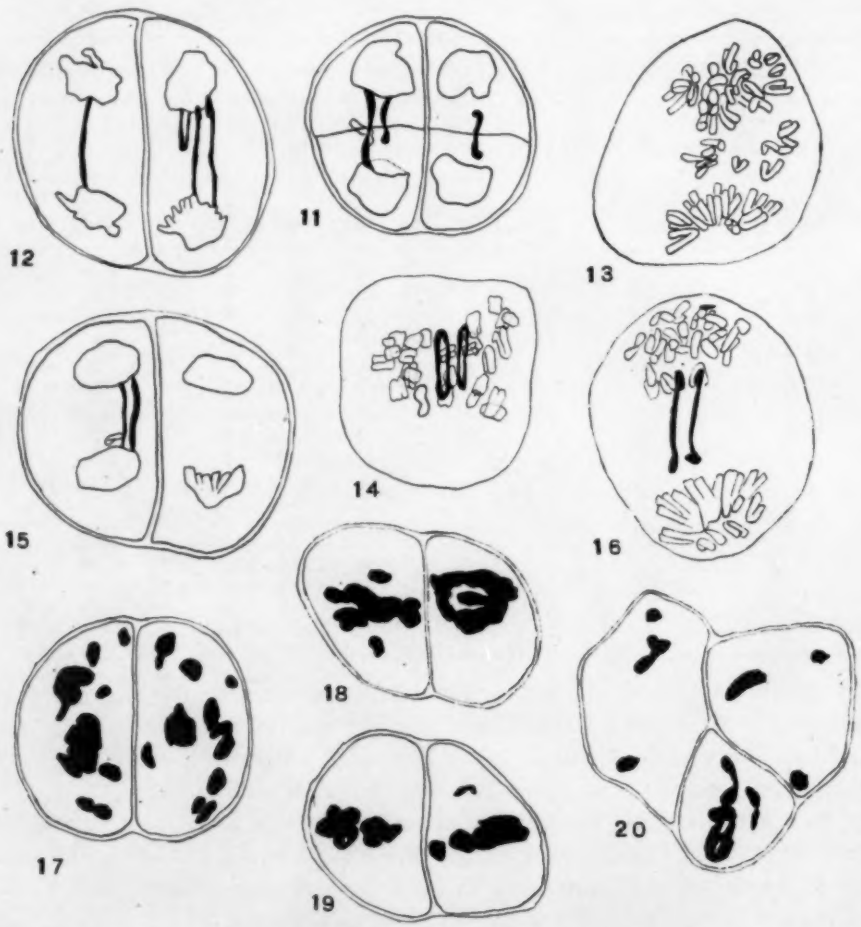
Number of microsporocytes in			Configurations					
T. macha × *T. turgidum*	*T. macha* × *T. durum*	*T. macha* × *T. dicoccoides*	I	II	III	IV	V	VI
.....	.....	1	5	10	.....	1	.....	1
1	1	.....	7	11	.....	.....	.....	1
1	.....	.....	9	10	.....	.....	.....	1
1	.....	.....	7	10	1	.....	1	.....
.....	1	.....	9	8	2	1	.....	.....
.....	.....	1	7	10	.....	2	.....	.....
2	.....	1	9	9	.....	2	.....	.....
.....	1	.....	4	12	1	1	.....	.....
.....	.....	1	6	11	1	1	.....	.....
1	.....	.....	5	12	2	.....	.....	.....
.....	.....	1	5	13	.....	1	.....	.....
4	1	2	7	12	.....	1	.....	.....
2	1	2	9	11	.....	1	.....	.....
1	.....	.....	11	10	.....	1	.....	.....
1	.....	.....	4	14	1	.....	.....	.....
.....	2	.....	6	13	1	.....	.....	.....
1	1	.....	8	12	1	.....	.....	.....
.....	1	.....	10	11	1	.....	.....	.....
4	7	1	7	14	.....	.....	.....	.....
1	4	1	9	13	.....	.....	.....	.....
20	.....	.....	7.60	11.80	0.25	0.55	0.05	0.10
.....	20	.....	7.55	12.65	0.35	0.20	0.00	0.05
.....	.....	11	7.27	11.45	0.09	1.00	0.00	0.09
Combined total: 51			7.51	12.06	0.25	0.51	0.02	0.08

the hexaploid hybrid, the chiasmata were mostly terminal—77.3 per cent terminal in *T. macha* × *T. turgidum*, 81.4 per cent in *T. macha* × *T. durum*, and 81.3 per cent in *T. macha* × *T. dicoccoides*. Interstitial chiasmata were again more variable in number than were terminal chiasmata.

In the pentaploids the number of univalents was negatively correlated with the number of chiasmata, values of −0.674 and −0.629 being obtained for *T. macha* × *T. turgidum* and *T. macha* × *T. durum*, respectively. These values are beyond the one per cent point of significance.

In the frequency of anaphase bridges the pentaploid hybrids differed strikingly from the hexaploids, in that bridges were much more frequent at second than at first anaphase (table 3). The three pentaploids averaged about five times as many microsporocytes with bridges at second as at first ana-

phase. This high frequency of second anaphase bridges appears not to be explainable on the basis of inversions. Whatever the cause of the bridges may have been, they apparently involved at least three different chromosomes in *T. macha* × *T. durum* (fig. 12) and at least two in *T. macha* × *T. turgidum* and *T. macha* × *T. dicoccoides* (figs. 11, 15). The bridges appeared to be confined to the long arm of chromosomes with subterminal centromeres, except in *T. macha* × *T. durum*, where chromosomes with submedian and median centromeres seemed to be involved also.



FIGS. 11, 12, 15. Bridges at second anaphase in (11) *T. macha* × *T. turgidum*, (12) *T. macha* × *T. durum*, and (15) *T. macha* × *T. dicoccoides*. × 500. FIG. 13. Normal anaphase I in *T. macha* × *T. dicoccoides*. × 500. FIG. 14. Multiple configurations delayed in division in *T. macha* × *T. durum*. × 500. FIG. 16. Two single bridges at first anaphase in *T. spelta* × *T. macha*. × 500. FIGS. 17–20. Crumbled chromosomes in *T. macha* × *T. dicoccoides*. × 500.

Long chains of three sometimes failed to congress in the pentaploids. The multiple configurations usually separated more slowly than the normal bivalents (fig. 14).

In *T. macha* × *T. dicoccoides* the first division was normal (fig. 13), but the chromosomes usually crumbled into masses at the second division (figs. 17–20). The abnormality occurred either before or after the formation of a metaphase plate. The chromatin masses formed varied considerably in number, size, and shape. This crumbling of chromosomes presumably accounts for the high percentage of sterility in this hybrid.

DISCUSSION

Little support is provided by the present investigation for the suggestion of Dekaprelevitch and Menabde (1932) that *Triticum spelta* may have been derived from *T. macha*. The irregular meiotic behavior and the low fertility of the *T. spelta* × *T. macha* F_1 certainly do not favor such a hypothesis; and neither does the fact that the spike of *T. macha* has a type of disarticulation not found in any variety of *T. spelta*.

The exact phylogenetic relationship of *T. macha* to the other species of hexaploid wheat is not at all clear. The fact that the intensity of pairing, as measured by the number of chiasmata, is essentially the same in the pentaploid hybrids as in *T. macha* itself suggests that the A and B genoms of *T. macha* may be identical with those of the emmers except for at least three reciprocal translocations (to account for the ring of six and association of four sometimes found in a single cell). This possibility of close relationship is favored by the relatively high fertility of two of the pentaploid hybrids—about 60 per cent, which is greater than ordinarily found in hybrids between *T. vulgare* and *T. durum* or *T. dicoccum* (Love 1941). Also, the meiotic irregularities of the pentaploid hybrids involving *T. macha* are no greater than in certain of Love's hybrids of *T. vulgare* with *T. durum*.

The possibility that *T. macha* may possess a somewhat different C genom than the other hexaploid wheats arises from the finding that pairing is relatively poorer in *T. spelta* × *T. macha* than in *T. macha* × the tetraploid wheats. Furthermore, the method of disarticulation of the spike is fundamentally different in *T. macha* from that in *T. spelta* or *T. vulgare*, and the type of awn is different from that in *T. spelta* and from the various types described by Watkins and Ellerton (1940) for *T. vulgare*. A simple explanation for these differences would lie in the independent origin of *T. macha* as an amphidiploid resulting from a cross of one of the emmers with a species of *Aegilops* having short awns (or lacking awns altogether) and a rachis fragile above the point of attachment of the spikelet.

SUMMARY

Hybrids with *Triticum spelta* and *T. vulgare* show that *T. macha* differs genetically and cytologically from the other hexaploid wheats. It has a dif-

ferent kind of awn and a different type of disarticulation of the rachis. The F_1 plants are very weak and are either completely sterile (*T. vulgare* × *T. macha*) or about 60 per cent sterile (*T. spelta* × *T. macha*). At meiosis *T. spelta* × *T. macha* showed an average of 2.90 chromosomes involved in multivalent configurations and 0.93 in univalents. Bivalents are of reduced chiasma frequency, and bridges are frequent at first anaphase.

Hybrids of *T. macha* with the tetraploid species *T. turgidum*, *T. durum*, and *T. dicoccoides* do not differ greatly from the similar hybrids involving *T. vulgare* or *T. spelta*. Multivalent associations occur, but bivalents are of approximately normal chiasma frequency. Anaphase bridges are found, particularly at the second division. Fertility is high (about 60 per cent) except in *T. macha* × *T. dicoccoides*, which is almost completely sterile, apparently through crumbling of the chromosomes at the second division. Awns are of intermediate length in these hybrids, and spikes are longer than in either parent.

DEPARTMENT OF AGRONOMY, COLLEGE OF AGRICULTURE AND FORESTRY
UNIVERSITY OF NANKING

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HETEROKARYOSIS IN PENICILLIUM NOTATUM

GLADYS E. BAKER

It has been recognized through experience that single-spore transfers of *Penicillium notatum* Westling are undesirable in maintaining an active penicillin-producing strain, for this practice is said to result in a less productive strain (Clutterbuck et al. 1932; Foster et al. 1943). Since the single-spore method is commonly regarded as means of securing pure lines in many fungi, it seemed important to investigate the nuclear behavior of this species to see if that might explain the necessity of mass spore transfers.

A penicillin-producing strain of this fungus (N.R.R.L. 1209) was used. Films of modified Czapek-Dox agar were spread on sterile slides and when dry were sown with a water suspension of spores from a seven-day culture on the same agar. The slides were incubated in sterile moist chambers at 25° C. Material was killed and fixed on the slides at intervals of 6, 12, 24, 36, 48, and 72 hours. Of the various fixatives employed, Bouin's and a modified F. A. A. gave the best results. Conidiophores with conidia frequently appeared as early as the 24-hour stage and 48 hours provided a full range of mycelial and conidial development. For nuclear stages of the conidia the spore suspension was put directly on slides with Mayer's adhesive, dried down lightly and immediately killed and fixed. In addition small portions of colonies on agar plates were cut out and fixed. These were embedded in paraffin and sections cut 7 μ and 10 μ thick. All slides were stained in Heidenhain's iron-alum-haematoxylin and counterstained with 1 per cent alcoholic phloxine. These agar-film slides were also used for microchemical tests. For direct observation of germination stages agar slide cultures were prepared after the method recommended by Henrici (1930).

The conidia of *P. notatum* are subglobose, smooth and of varying diameters. Before germination they range from 2.0–4.0 μ in diameter. Microchemically the wall substances give a positive reaction for cutin and pectin, but not for cellulose; for storage substances they give positive tests for glycogen, fats, and proteins. Their enlargement prior to germination is conspicuous, often doubling their size. Conidia produced in the same chain frequently do not separate before they germinate. The wall between two such contiguous cells is conspicuously thinner. A germ tube may be produced from each of these conidia independently, or less commonly from only one of the spores, in which case the nucleus of the second spore may migrate into it (fig. 2).

A conidium typically contains one nucleus and germinates by a single germ tube, or less frequently by two germ tubes (figs. 1, 3). The nucleus

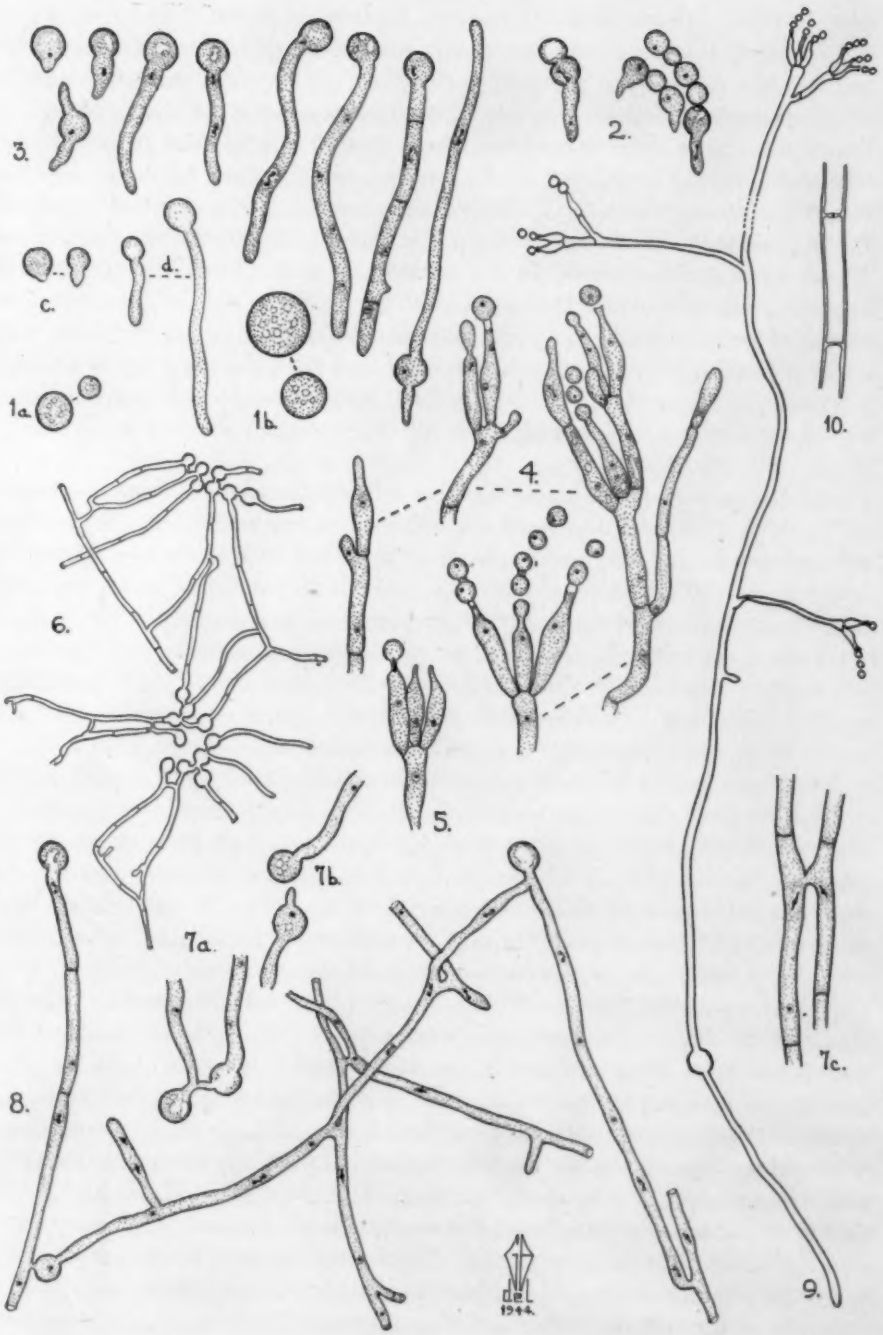
divides as the germ tube is produced. One of the two daughter nuclei may remain in the conidium or near it, the second one moving out into the hypha for further division. Or both the daughter nuclei may move out into the elongating hypha and divide again. Germination of the spores occurs in 6–7 hours after sowing and in two more hours the hypha may be 50 μ long and in the 4-nucleate stage (fig. 1, *c*, *d*). Occasionally one finds two nuclei in a conidium but they often evince signs of recent division, which suggests that the binucleate condition arose subsequent to separation from the conidiophore. No conidia were found with more than two nuclei. Many nuclear divisions accompany the elongating hyphae. Cross walls may or may not follow these early mitotic divisions. As the hyphae elongate the more distal portions are clearly septate. In the basal portions (nearer the original spore) the nuclei may never become separated by walls, but they are spaced well apart. The mycelial cells are consistently uninucleate in the distal septate regions; elsewhere they may be binucleate or even plurinucleate through intercalary division and lack of immediate septation.

Conidiophores are produced early and freely by the mycelium. The phialides are uninucleate with regular nuclear divisions which provide daughter nuclei for the developing conidia. The first conidium of a series is formed at the apex of the phialide as a swollen vesicular expansion, the connection between it and the phialide gradually contracting so that only a narrow connective remains at maturity, through which the nucleus passes in an attenuated fashion. Once within the spore the nucleus again becomes globose (fig. 5). Successive conidia seem to be formed by continued enlargement and constriction of the phialide behind the first-formed conidium. The process is accompanied by continued mitotic division of the producing phialide.

There is nothing unusual about this behavior. However, in watching the progress of germination one finds that after 12 hours or so the frequency of anastomoses involving the spores and their germ tubes is very striking. Such anastomoses are not unnoted for the *Penicillia* but in connection with their nuclear history may be important. Anastomoses occur between spores by short hyphal connectives, between spores and hyphae, and between ma-

Explanation of figures 1–10

FIG. 1*a*. Spores before germination. × 1850. *b*. Spores at germination. × 1850. *c*. Spores at seven-hour germination stage. × 590. *d*. Spores and hyphae at nine-hour stage. × 590. FIG. 2. Contiguous conidia germinating. × 1850. FIG. 3. Germination stages showing nuclei. × 1850. FIG. 4. Stages in conidiophore and conidia formation. × 1850. FIG. 5. Nucleus passing into first conidium. × 1850. FIG. 6. Germinating and anastromosing spores at 12 hours. × 390. FIG. 7 *a*, *b*, *c*. Types of anastomosis. × 1850. FIG. 8. Detail of eight variously derived hyphal strands and their anastomosis. Not all of the conidial sources are illustrated. × 900. FIG. 9. Hyphae and conidiophores from a single spore, 48 hours. × 390. FIG. 10. Septal pores between cells. × 1850. All figures drawn with the aid of an Abbé camera lucida.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ture hyphae, producing a microscopic picture of much interlacing (figs. 6, 7). The total effect is of large clumps of spores as foci from which radiating hyphae fuse freely amongst themselves (fig. 8). Nuclear descendants of many spores thus have possible introduction into the mature hyphae. In figure 8 at least eight spores and their hyphal outgrowths are confluent. The nuclei divide very freely and their passage from one hypha to another is easily demonstrated (fig. 7). This free movement in the hyphae is greatly facilitated by the infrequency of septa in the oldest regions of the mycelium (those nearest the spores). In the septate areas the nuclei possibly could migrate from cell to cell through septal pores. These can be demonstrated by the chlor-zinc-iodide and I_2KI method, although they are very fine and difficult to show (fig. 10). Any significance that might be attached to a plurinucleate condition of the conidia is diminished when it is apparent that a random assortment of nuclei from various conidial sources is so easily obtained in the hyphae.

Each uninucleate conidium receives a daughter nucleus from the original nucleus of the phialide, itself a descendant of any nucleus in the mycelial network producing the conidiophore. If there are any factorial differences in the nuclei of the mycelium their allotment to the conidium-producing cells is certainly random as a result of anastomoses, and consequently conidia from the same fertile hypha could carry different nuclear factors. Likewise if a single conidium with a single nucleus is germinated to produce mycelium and conidiophores it is obvious that the first conidial descendants all have nuclei exactly alike, borne on a mycelium without anastomoses. That a single spore if germinated by itself can produce conidiophores and conidia is not difficult to show (fig. 9). Subsequently these conidia in turn will germinate to increase the colony by more hyphae which then may anastomose. However, the nuclei will all be identical, unless somatic mutation occurs. In watching individual spores widely spaced on agar films, it was evident that these spores with no chance to develop anastomoses germinated much more slowly and less vigorously than those sown in large numbers.

A survey of the literature reveals very few cytological descriptions of *Penicillium*. Thom (1930) recognizes the phenomenon of anastomosis in the genus and cites Dangeard's work on the cytology of certain species. The identity of Dangeard's species may not be clear today, but there can be no doubt that as early as 1907 he described a *Penicillium* (*P. vermiculatum*) with uninucleate spores and mycelium (see his plate 17, figures 10 and 11), and that this species also exhibited frequent anastomoses. He describes the fusion of the anastomosing cells, the resorption of the walls at those points, and the subsequent nuclear passage. In another species (*P. crustaceum*) he notes that the thallus cells are plurinucleate, but that they become uninucleate in the conidiophore. Conidium formation he likens to the budding

of yeasts, each conidium receiving a daughter nucleus prior to the formation of a basal wall. Dangeard's papers still represent the most complete cytological series available.

Anastomoses among germinating hyphae in *Penicillium* were recognized in 1893 by Wehmer who described them for *Penicillium luteum*. Anastomosis in other Ascomycetes has been noted as early as 1870 by de Bary and Woronin in two species of *Sordaria*. The Tulasne brothers illustrated the same behavior in *Cryptospora aucta*. Their comments have several points of interest in connection with *Penicillium notatum* and its anastomoses. The Tulasnes mentioned that those germ tubes which had been growing for a long time formed septa and that the young germ tubes coalesced with each other or with the conidia themselves so that "wonderful anastomoses" arise (Grove *et al.* 1931). Biourge (1923) illustrates various species of *Penicillia* without particular reference to their nuclei. Some of his figures of spores and cells suggest that these are uninucleate,—one may be binucleate—but no such statement attends the figures. Probably there are both uninucleate and plurinucleate species of the genus.

Some species of *Penicillium* produce an ascocarpic or zygotic stage, but this type of reproduction is unknown for *P. notatum* Westling, and consequently the combination and segregation of hereditary factors through karyogamy and meiotic division cannot be shown by genetic experiments as it can in some fungi (e.g., *Venturia inaequalis,* Keitt 1941; *Hypomyces solani* f. *cucurbitae,* Hansen and Snyder 1943). However, beginning with the work of Hansen and Smith in 1932 there has been serious consideration of the problem of variation in imperfect fungi. Hansen has introduced the term "dual phenomenon" to explain the means of variation in these azygotic fungi. In 1938 Hansen described the results of single-spore isolations from "multinucleate, binucleate, and mainly uninucleate" spores. The last category is the one in which *P. notatum* would fall. From his multinucleate single-spore cultures he found three types of variants were produced: two contrasting types, the homotypes, and their intermediate types, the heterotypes. The latter consistently give rise to more heterotypes and the former reproduce themselves. This dual phenomenon is due to heterokaryosis, or the possession of two genetically unlike kinds of nuclei in the cells. Their separation results in homotypes. It is easy to see how this operates in multinucleate spores—the phenotypic expression of the dual phenomenon is the result of the different genotypic nuclei present in them. If all the nuclei in a spore are genotypically identical then a homotype results. Differences in balance between the two nuclear types gives different phenotypic heterotypes. In uninucleate lines Hansen also isolated two homotypes (*Verticillium alboatrum*) but it took many cultures—up to 50—to produce a line with the two homotypes together in the same spore. He assumed that these spores then are

binucleate. Consequently in maintaining such a fungus by single-spore transfer the chances of selecting a spore containing both homotypes is extremely small. Unless that particular kind of a spore is transferred the subculture will carry only one of the homotypes. Hansen feels that the occurrence of the dual phenomenon is frequent enough to warrant regarding it as the natural condition in imperfect fungi.

Post (1933) from single-spore cultures of *Macrophomina Phaseoli* derived two lines, one with and one without pycnidia. In *Penicillium notatum* the particular factorial difference under consideration is physiological: the penicillin-producing capacity. The means by which heterokaryosis is established in this fungus have already been shown. The cytogenetic behavior easily allows for the presence or absence of differing factors in the mycelium and consequently among the spores. Hansen (1942) remarks that heterokaryosis cannot occur in uninucleate cells. Such a conclusion is obvious, but if the nuclear behavior is such as to allow for genetic differences among spores even if not in them, then the ultimate effect achieved through subsequent anastomoses of the hyphae from these spores is the same. To support this reasoning a quotation from Hansen (1942) seems appropriate: "In this connection it is well to remember that at least two factors or mechanisms operate against the possibility of the uninucleate condition being continuous or permanent in any fungus. Those two factors, mitosis and anastomosis, operating singly or together are able to produce binucleate, trinucleate, and quadrinucleate cells in fungi generally considered to have uninucleate cells."

In *Penicillium notatum* the random assortment or mixing of nuclear types in the mycelium produces the effect of heterokaryosis even though the septate portions of the thallus are uninucleate. Dodge (1942) has discussed the effect of the inclusion of different sets of genes in the same cytoplasm but not in a common nucleus. The dissociation of homotypes from the dual condition can be concerned with physiological or morphological differences. Their association produces heterokaryotic vigor.

Therefore the trouble with single-spore transfers of *Penicillium notatum* is due to the fact that such practice does carry on a pure line but presumably a homotypic and not a heterotypic one. And the maintenance of the incomplete nuclear line is undesirable. By perpetuating the homotypes together or assuring heterocaryosis, the desired strain can be maintained. In *Penicillium notatum* this is done easily by mass spore transfer which assures an abundance of anastomosing hyphae and a recombining of the homotypes.

SUMMARY

1. Conidia of *Penicillium notatum* West. are uninucleate. They germinate by one or two germ tubes.

2. Anastomosis occurs freely among spores and hyphae, accompanied by nuclear division and nuclear migration.

3. The assimilative hyphae are plurinucleate or binucleate, becoming uninucleate in the cells subtending the conidiophores. Phialides are uninucleate.

4. The anastomosing of hyphae and spores in multiple spore transfers assures a combining of factors and heterokaryosis as spores with nuclei of different origins are brought together.

PLANT SCIENCE DEPARTMENT, VASSAR COLLEGE
POUGHKEEPSIE, NEW YORK

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BULLETIN OF THE TORREY BOTANICAL CLUB
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BRAZILIAN CHYTRIDS—I. SPECIES OF NOWAKOWSKIELLA

JOHN S. KARLING

While serving as Field Director of the exploration department of the U. S. Government's Rubber Development Corporation in Brazil during 1943, the author had unlimited opportunities to collect soil and water samples for study of the aquatic fungi of the Amazon Valley. Inasmuch as nothing is known about the aquatic fungi of this vast tropical area, it seemed highly worth while to the writer to utilize this opportunity, although he was primarily engaged in exploring the Brazilian jungles for untapped sources of wild rubber. Soil and water samples were collected from swamps, small streams, lakes, and rivers in various localities and sent to central headquarters in Manaus, Amazonas, where they were baited with bits of young corn leaves, thin onion skin, cellophane, hemp seeds, and other favorable substrata. These samples were examined and studied in a preliminary manner at Manaus as time and opportunity permitted and were later sent to Columbia University for further culture and intensive investigation.[1]

Among the numerous chytrids discovered and cultured in this manner were five species of *Nowakowskiella: N. ramosa, N. elegans, N. profusa,* and two additional species which appear to be new. The first of these new species is distinguishable by an unusual type of sporangium dehiscence and by zoospores which contain numerous small granules instead of a single large refractive globule like other species of *Nowakowskiella.* For this reason it is named *N. granulata.* The second species is characterized primarily by elongate, clavate, cylindrical, septate or non-septate sporangia in addition to pyriform and spherical ones, and is accordingly designated as *N. elongata.*

Nowakowskiella granulata Karling, sp. nov. Fungus saprophyticus; rhizomycelio profuso, copiose ramoso, juveni hyalino in aetate paulo fuscante et membranum crasso, partibus tenuibus 1.5–7 μ diametro, interdum reticulatis, incrementis pluribus plerumque non septatis, ovalibus 6–8 × 9–11 μ, vel late fusiformibus 5–9 × 8–13 μ, vel prope globosis 6–10 μ diametro, vel irregularibus; sporangiis terminalibus intercalaribusve, plerumque non apophysatis, globosis 12–35 μ diametro, vel piriformibus 12–22 × 15–30 μ, vel ovalibus 10–18 × 12–25 μ, vel interdum irregularibus, 1–3 papillas efferentes 3 × 5 μ, vel 1 plurave collos elongatos habentibus; papillae efferentis apice canaliculisve maturis mollescentibus obturaculum opacum in ore facientibus, protoplasmati inter haec contracto et operculum sub obturaculo

[1] The writer is deeply grateful to Miss Anne M. Hanson and Miss Alfhild Johanson for receiving the water and soil samples and taking care of the cultures during his absence in Brazil.

faciente; operculis hypocrateriformibus cyathiformibusve, semiglobosis conicisve, 3–7 μ diametro; zoosporiis globosis 5–6.6 μ diametro, plura aureo-brunea grana capientibus, flagello circa 35 μ longo; sporis perdurantibus levibus hyalinis, membranum 1.5–2 μ crassis, globosis 15–24 μ, vel ovalibus 15 × 20 μ, globulum refringens magnum (ad 12 μ diametro) cum pluribus parvis habentibus; germinatione non visa.

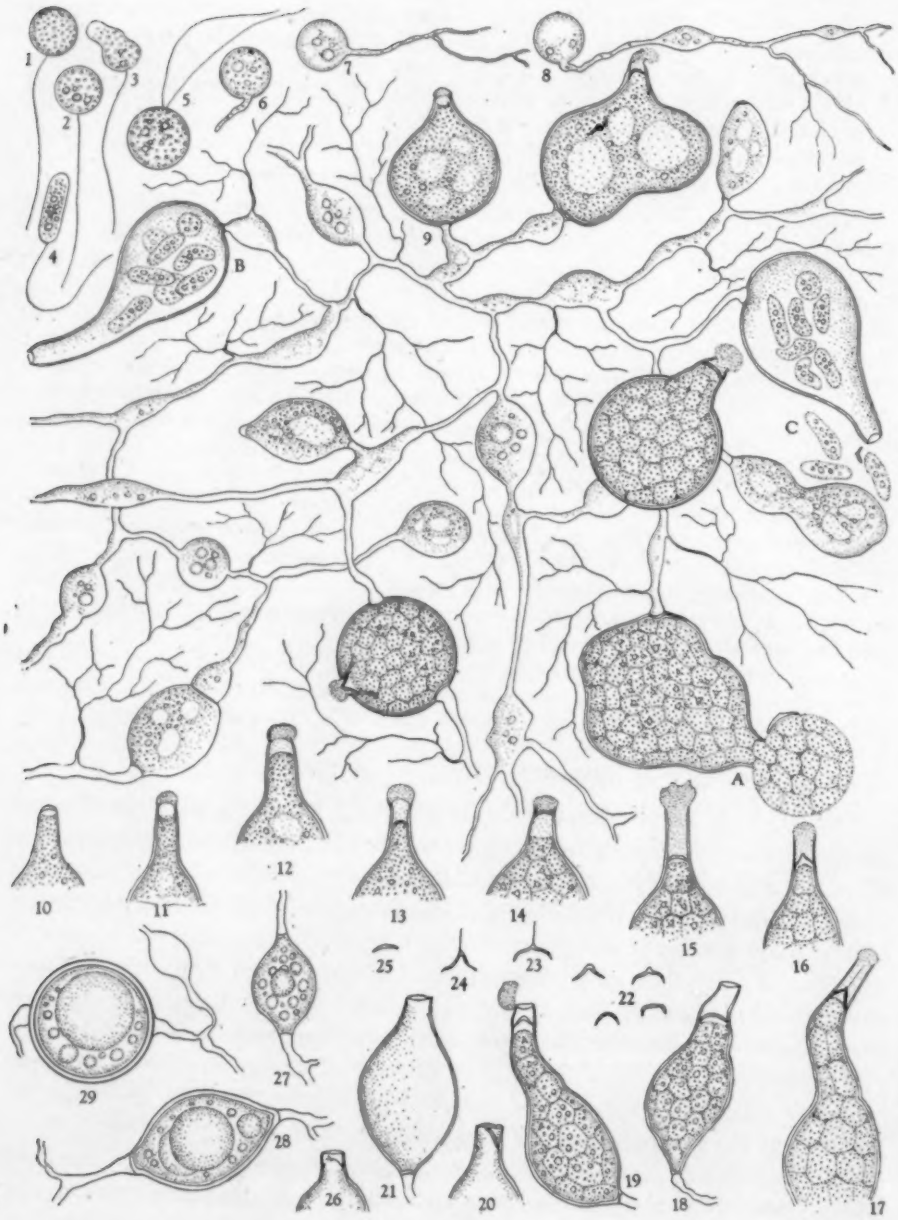
Saprophytic in decaying vegetable debris in Igarape San Carlos, Matto Grosso and Igarape Velho near Porto Velho, Amazonas, Brazil.

Nowakowskiella elongata Karling, sp. nov. Fungus saprophyticus; rhizomycelio hyalino, profuso, copiose ramoso, partibus tenuibus 1–6 μ diametro, interdum reticulatis, incrementis pluribus non septatis, ovalibus 5–13 × 7–15 μ, vel late fusiformibus 4–8 × 8–17 μ, vel globosis 5–15 μ, plerumque multos globulos refringentes capientibus; sporangiis terminalibus intercalaribusve, plerumque non apophysatis, septatis vel non, longe clavatis, 8–40 × 20–820 μ, vel cylindricis apicem turgidis, curvis complicatisve rectisve, 5–20 × 30–900 μ, vel piriformibus 15–44 × 20–70 μ, vel globosis 10–70 μ diametro, vel ovalibus irregularibus; operculo circulari ovalique in extrema linea, hypocrateriformi, 4–8 μ diametro, rare permanente; zoosporiis globosis, 5–6 μ diametro, unico (rare duobus) globulo refringenti conspicuo 2–2.5 μ diametro habentibus; sporis perdurantibus plerumque intercalaribus ex incrementis ortis, hyalinis levibus globosis, 16–24 μ diametro, vel ovalibus, 14–16 × 18–22 μ, magnum globulum refringentem a globulis parvioribus tabulatim circumdatum habentibus, germinantibus ut prosporangiis, parte interiori emergente ut zoosporangio membranam tenui ad superficiem sporae.

Saprophytic in decaying vegetable debris from Rio Negro and swamps at Flores Nabuco near Manaus, Amazonas; in soil samples from Campo Grande, Matto Grosso, and Rio Madeira near Porto Velho, Amazonas, Brazil.

Nowakowskiella granulata

As has been noted elsewhere, this fungus differs primarily from other species of *Nowakowskiella* by its unusual type of sporangium dehiscence and granular content of the zoospores. As is shown in figure 1, the latter are spherical and lack the single large refractive globule which is so characteristic of other species of this genus. Instead, the refractive material is usually dispersed in the form of numerous small golden-brown granules of fairly uniform size, which impart to the protoplasm a typical granular appearance. Sometimes, however, the granules may coalesce and form several larger refringent globules (figs. 2–5). The method of emergence from the sporangium (fig. 9 A), subsequent behavior at the exit orifice, and type of motility of the zoospores, on the other hand, are essentially similar to those of other species of *Nowakowskiella*. They may frequently become amoeboid (fig. 3) and creep about as elongate rods (fig. 4). Such shapes are common for spores remaining in the sporangia after the latter have opened (fig. 9 B, C). Large biflagellate zoospores are occasionally formed as the result of unequal or incomplete cleavage (fig. 5).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The type of zoospore germination (figs. 6–8) and the development of the thallus are fundamentally similar to those of other species of *Nowakowskiella* and *Cladochytrium* described by Berdan (1941), Hillegas (1941), and other workers and need not be emphasized in detail here. It is to be noted, however, that as the zoospores come to rest and germinate, the refractive granules aggregate and coalesce to form from two to seven larger refringent globules. Figure 9 shows a portion of the rhizomycelium of *N. granulata* with its spindle organs, rhizoids, and sporangia in various stages of development and maturation.

Particularly different from other species, however, is the manner of sporangium dehiscence and the changes involved in operculum development. In all other known members of *Nowakowskiella*, the operculum is formed at the tip of the exit papilla or canal and is pushed up as the zoospores emerge, whereas in *N. granulata* it is usually formed at varying depths within the papilla or exit canal. The successive stages of this process are shown in figures 10–19. In the early stages (fig. 10) the tip of the exit tube looks like that of any other operculate species, but within a few hours it begins to soften and swell (fig. 11) so that a plug of opaque material is eventually formed at the apex. At the same time the more granular and heterogeneous protoplasm in the neck usually recedes downward, leaving a hyaline area of varying size beneath the plug. This area appears to be filled with a hyaline, more or less viscid substance. At first the surface of the granular protoplasm in the tube is concave (fig. 11), but with further development it becomes convex (fig. 12). Figure 12 shows a later stage in which the opaque plug has increased in size, while the granular protoplasm has receded further and become convex on its surface. Sporangia in this stage of development are strikingly similar to those of *Cladochytrium hyalinum* and *C. crassum* described by Berdan (1941) and Hillegas (1941)—so much so that, until the formation of the operculum had been observed, the author regarded the

Explanation of figures 1–29

Nowakowskiella granulata. FIGS. 1–4. Spherical, amoeboid and elongate zoospores with finely granular contents. × 3100. FIG. 5. Giant bifiagellate zoospore. × 3100. FIG. 6–8. Germination of zoospores. × 3100. FIG. 9. Portion of the rhizomycelium showing developmental stages of the sporangia. × 2800. FIGS. 10–14. Successive stages in the deliquescence of the tip of the exit tube, formation of a plug of opaque substance, and the development of sunken opercula. × 2800. FIGS. 15, 16. Plugs of opaque substance extending down into the tube to sunken opercula. × 2800. FIG. 17. Longitudinal section of sporangium with a cone-shaped operculum capped by a fine filament. × 2800. FIG. 18. Section of a sporangium with empty exit canal above operculum. × 2800. FIG. 19. Plug of opaque substance displaced and adhering to tip of exit canal. × 2800. FIG. 20. Empty sporangium showing region of attachment of operculum in exit canal. × 2800. FIG. 21. Persistent operculum in exit canal. FIGS. 22–25. Variations in size and shape of opercula. × 3100. FIG. 26. Sporangium with a very thin, frayed, persistent operculum. × 2800. FIG. 27. Early stage of resting spore development. FIGS. 28, 29. Mature resting spores. × 2800.

present fungus as a species of *Cladochytrium*. In figure 13 an increase in size of the opaque plug and further recession of the protoplasm are evident. This figure also shows the first indication of operculum development at the surface of the receded granular protoplasm in the form of a cup-shaped thickened layer. Apparently further thickening of the bounding or plasma membrane occurs until a well defined operculum is formed (figs. 14–18).

Slight variations of the above described process often occur. Recession of the granular protoplasm in the neck may be very slight or even lacking so that the operculum develops immediately beneath the plug (fig. 14). In such instances recession may sometimes take place after the operculum is formed with the result that the hyaline area may occur between the operculum and sporeplasm. Sometimes the opaque substance may extend down into the exit tube to the operculum, as is shown in figures 15 and 16. In a few sporangia observed the opaque material had apparently deliquesced completely, leaving the exit tube empty above the operculum (fig. 18). In two instances the plug was uplifted more or less intact, displaced, and attached to one side of the exit canal (fig. 19), although the zoospores and sunken operculum had not emerged. Normally, however, the operculum and plug are pushed out simultaneously as the zoospores emerge.

As has been noted in the diagnosis above the opercula are oval or circular in outline and shallow saucer-, bowl-, cup-, and cone-shaped (figs. 22–25). Sometimes they may be distinctly apiculate (fig. 16) with the apex capped by a tenuous spine of hyaline material (figs. 17, 23, 24). Oftentimes the operculum does not separate flush with the inner wall of the exit canal but leaves an inward projecting ring of wall material, which in longitudinal section looks like two short sharply-pointed ledges (fig. 20). A few sporangia have been observed in which the operculum was persistent inside the exit tube (fig. 21). Figure 26 shows a sporangium with an operculum which is very delicate, thin and frayed at the edge. In such cases the operculum may be so thin that it can be seen only with difficulty, and the sporangia accordingly appear at first sight to be inoperculate.

The occurrence of such poorly defined opercula and the presence of the *Cladochytrium*-like plugs of opaque material in the exit orifices (figs. 12–17) suggest the possibility that an evolutionary transition from inoperculate cladochytriacous species to operculate ones or vice versa may have occurred through dehiscence changes of the nature described above In that event *N. granulata* may possibly be regarded as a transitional species.

Only a few resting spores have been observed in *N. granulata*. They are usually formed from the intercalary swellings which increase in size, develop an abundance of refractive substance (fig. 27) and become thick-walled. With further development, most of the refringent globules coalesce and form one or two large central ones which may be surrounded by a layer of

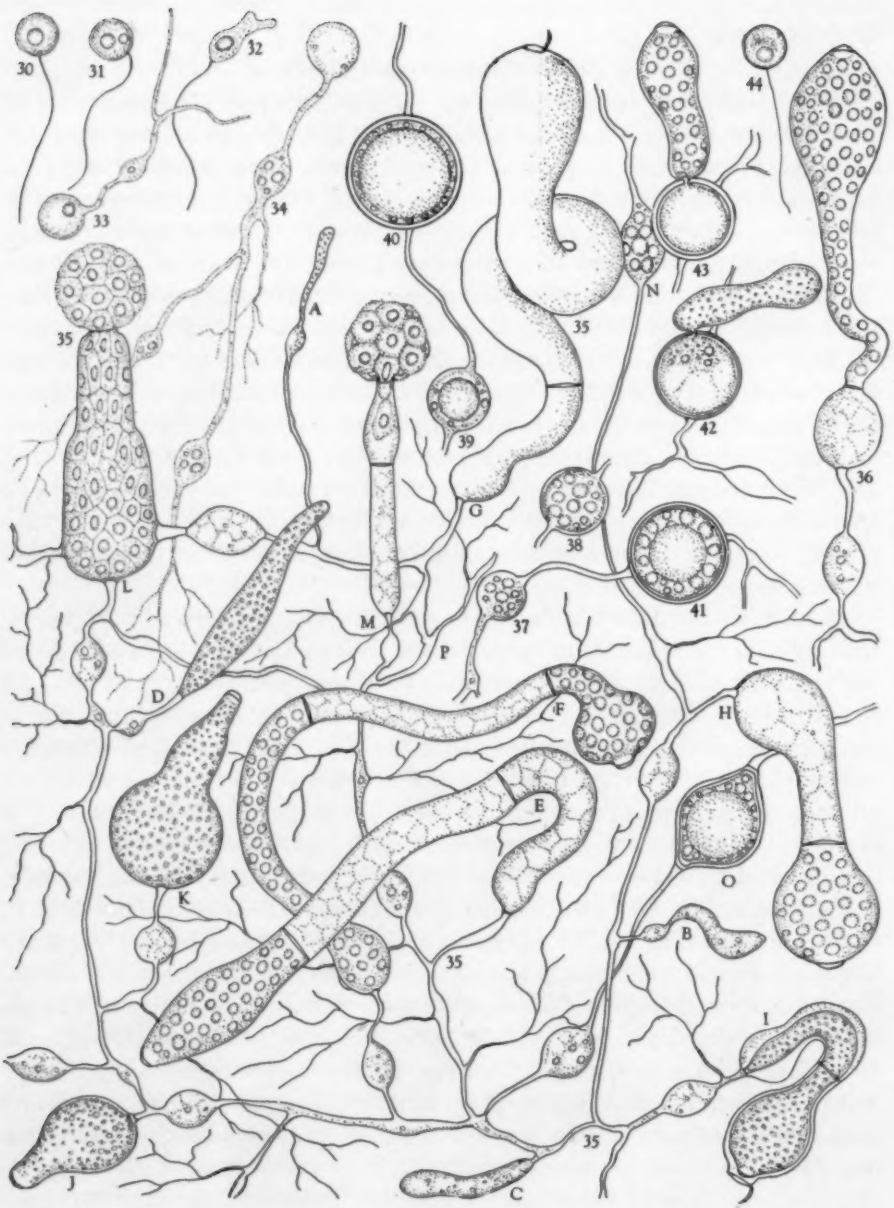
smaller globules. As is shown in figures 28 and 29, the resting spores may be oval, citriform, and spherical in shape. So far, no germination stages have been observed.

NOWAKOWSKIELLA ELONGATA

This species has been collected in the northern and southern parts of Matto Grosso and in Amazonas near Manaus in moist soil as well as water samples. It usually crops up in great abundance in cultures baited with corn leaves and onion skin, and as far as present observations go, it appears to be more widely distributed and ubiquitous than *N. granulata*. Its essential structural characters and developmental phases are illustrated in figures 30–43. Except for the sporangia, the rhizomycelium of *N. elongata* resembles more closely that of *N. elegans* than any of the other species of *Nowakowskiella*. Its zoospores are of the same size and contain one or rarely two conspicuous refractive globules (figs. 30–32). They may likewise become amoeboid (fig. 32), creep around, and then round up and dart off. No fundamental differences in zoospore germination (figs. 33, 34) and development of the rhizomycelium have been observed in this species, and consequently it would be superfluous to describe these developmental phases in detail. The present description will accordingly be confined to the principal structural differences.

A portion of the rhizomycelium with its variously-shaped sporangia, broadly oval spindle organs, rhizoids, and resting spores is shown in figure 35. The most striking structures in this figure are the elongate, clavate and cylindrical sporangia (figs. 35 E, F, G, H, I, 36). These sporangia are so similar to those of *Cylindochytridium* (Karling 1941a) that, until the polycentric rhizomycelium had been observed, the author believed that they were related to this monocentric genus. As was noted earlier, the sporangia may be straight, curved, or coiled, non-septate or with one to three septa. The apical end in which the spores are formed is usually inflated or swollen. The base of the sporangium may also be slightly inflated, as is shown in figures 35 F, G, H, I, and 36. Figure 35 F shows an exceptional sporangium in which the zoospores were formed in both the basal and apical ends with a sterile vacuolate segment in the center. Not all sporangia, however, are elongate, clavate, and cylindrical. They may also be spherical, pyriform, flask-shaped, and irregular. Figures 35 J and K show two pyriform sporangia, while the one illustrated in figure 35 L is intercalary and flask-shaped with rhizoids arising from the sides as well as the base. In contrast to those of *N. elegans*, these sporangia are rarely apophysate. Instead, they are usually subtended by a short tenuous filament which leads to an intercalary swelling or spindle organ (figs. 35 F, I, 36).

Stages in the development of the elongate sporangia are shown in figures 35 A, B, C, and D. Such sporangia usually begin as elongate clavate swell-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ings at the tips of the rhizomycelium (fig. 35 F) which increase in length and diameter (figs. 35 B, C) and are soon delimited by a cross septum from the remainder of the thallus (fig. 35 D). At first the protoplasm is dense and greyish-granular, but as development progresses, vacuoles appear and the refringent granules gradually coalesce into larger globules. As the sporangium elongates and the denser protoplasm gradually moves toward the apex, the basal portion becomes more and more vacuolate and is eventually delimited by a cross wall. If further elongation occurs, the process is repeated along the length of the sporangium, and secondary and tertiary septa are formed. In the sporangium shown in figure 35 F movement of the denser protoplasm was apparently toward both ends, so that the central portion became vacuolate and was cut off by cross walls. The dense protoplasm in the apex soon undergoes cleavage into zoospores which eventually push off the operculum, emerge, and form a globular mass at the exit orifice (figs. 35 L, M). The subsequent behavior of the zoospores is essentially similar to that of other species of *Nowakowskiella*.

Resting spores occur rather commonly in old cultures of *N. elongata*, and as in the previously described species, they are usually formed by the growth and transformation of spindle organs. The early developmental stages may be easily recognized by an increase in the number and size of the refractive globules in the intercalary enlargements (figs. 35 N, 37). This increase continues as the enlargements grow in size (fig. 38), and gradually the globules coalesce to form a large central or excentric one. The wall continues to thicken in the meantime until the large oval, citriform, or spherical resting spore is fully formed (figs. 35 O, 40, 41). Particularly conspicuous is the size of the larger refractive globule in the mature spore. As has been noted elsewhere, it may be up to 15 μ in diameter and in some cases almost completely fills the spore. When smaller in size, it is usually surrounded by one or two layers of small globules (fig. 41).

So far, only a few germination stages have been observed. In this process the content of the spore emerges through a pore in the wall and forms a thin-walled evanescent zoosporangium on the surface as in other chytrid species. Figures 42 and 43 show late progressive developmental stages of small elongate sporangia, but more elongate and septate sporangia also have been observed.

Explanation of figures 30–44

Nowakowskiella elongata. FIGS. 30, 31, 32. Spherical and amoeboid resting spores. × 3100. FIGS. 33, 34. Germinating spores. × 3000. FIG. 35. Portion of the rhizomycelium showing variations in the shape and structure of the sporangia. Hyphal anastomosis at *p*. × 2800. FIG. 36. Clavate sporangium with inflated base. FIGS. 37–39. Stages in the development of resting spores from spindle organs. × 3000. FIGS. 40, 41. Mature resting spores. × 3000. FIGS. 42, 43. Germinating resting spores. × 2800. FIG. 44. Zoospore from germinated resting spore. × 3100.

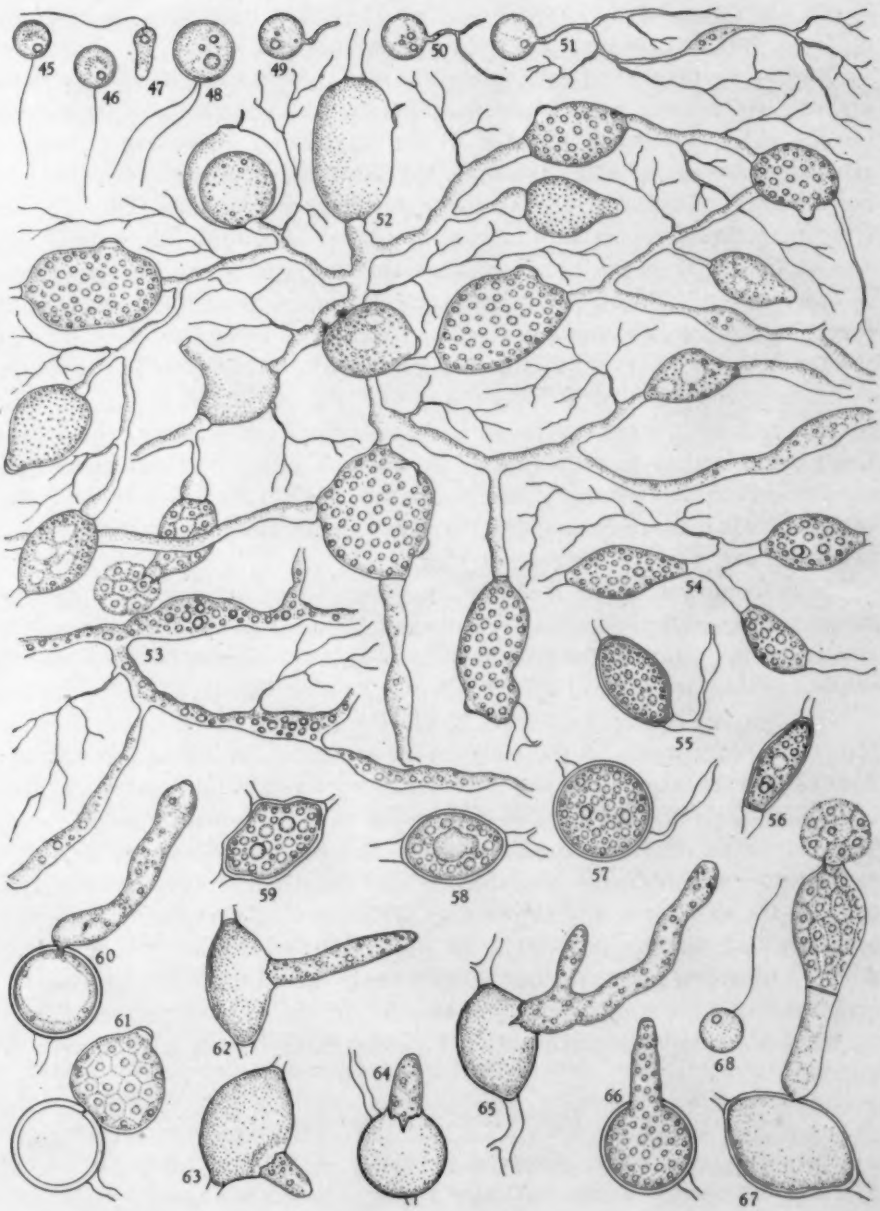
NOWAKOWSKIELLA PROFUSA

This species was discovered by the author in New Kent County, Virginia, in the winter of 1941, and later (1941a, 1942) collected in Texas, Alabama, Arkansas, Florida, Georgia, Louisiana, Mississippi, South Carolina, and Tennessee. It appears thus to be widely distributed in the southern states, and consequently it was not surprising to discover its occurrence in South America also. So far, however, it has been found in only one locality in Brazil—in a moist soil sample at San Carlos on the Madeira-Mamore railroad in Matto Grosso.

Inasmuch as *N. profusa* has been only briefly described and never illustrated, a short description with figures of its chief structural characters and developmental stages is herewith presented from the Brazilian material. No fundamental differences between the South American and North American material has been found, so that the present account holds for both types. *Nowakowskiella profusa* differs principally from the other known species of this genus by its smaller zoospores, the rare occurrence or even lack of well-defined spindle organs, and yellowish-brown resting spores which may function directly as sporangia with exit canals in germination or act as prosporangia and give rise to thin-walled zoosporangia on their surface. The comparatively smaller zoospores and their more minute refractive globules are illustrated in figures 45–47. They germinate (figs. 49–51) and give rise to a tenuous rhizomycelium in the same manner as in the previously described species. The fully developed and mature thallus is relatively coarse, and has the appearance and structure of the portion shown in figure 52. An abundance of intercalary oval, elongate, and spindle-shaped sporangia with one or more apical, subapical, or lateral opercula is common and distinctive in this species. Particularly characteristic is the rare occurrence per se or lack of sharply defined relatively empty spindle organs of the type found in *N. granulata, N. elongata,* and other cladochytriacous species. Intercalary swellings frequently develop, but they usually increase in size and become sporangia or resting spores. In a previous publication (1941a) the author reported that the tenuous portions of the rhizomycelium vary from 6 to 15 μ

Explanation of figures 45–68

Nowakowskiella profusa. FIGS. 45–47. Spherical and amoeboid zoospores with single small refractive globule. × 3100. FIG. 48. Giant biflagellate zoospore with three refractive globules. × 3100. FIGS. 49–51. Germination of zoospores. × 3100. FIG. 52. Portion of the rhizomycelium. × 2800. FIG. 53. Portion of an older rhizomycelium with numerous refractive globules. × 300. FIG. 54. Early stages in resting spore development. × 2800. FIGS. 55–59. Variations in size and shape of resting spores. × 2800. FIGS. 60, 61. Longitudinal views of resting spores which are functioning as prosporangia in germination. × 2800. FIGS. 62–65. Surface views of resting spores which are germinating directly as sporangia with exit tubes. × 2800. FIG. 66. Similar resting spore in longitudinal view. × 2800. FIG. 67. Septate sporangium from germinated resting spore with emerging zoospores. × 2800. FIG. 68. Zoospore from germinated resting spore. × 3100.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in diameter. Subsequent study of additional North American as well as South American material has shown that the larger diameters are very exceptional and that the more normal range is from 1 to 9 μ.

Resting spores occur very abundantly in *N. profusa* and germinate after a short rest period. As cellophane and corn leaf cultures of this species become older, a marked increase in the amount of refractive substance usually occurs in the rhizomycelium (fig. 53). This change is usually, but not always, indicative of resting spore development. In such thalli the refringent globules accumulate in the intercalary swellings, which later become delimited from the more tenuous empty portions of the rhizomycelium by cross walls (fig. 54). These segments are the incipient resting spores. With further growth and increase in size, aggregation of refractive globules, and thickening of the wall they finally attain maturity. Unlike those in the spores of the previously described species, however, the refringent globules rarely coalesce to form a large central one. Instead, they usually remain comparatively small and evenly distributed (figs. 55–57, 58). With increasing age, the wall of the spore turns yellowish-brown in color. As has been reported earlier (1941a), the mature spores may be elongate, fusiform, truncate, oval, spherical, and slightly irregular in shape.

In germination, as noted earlier, they may function as prosporangia or directly as sporangia. In the former case, the content emerges through a relatively small pore in the wall and develops into thin-walled zoosporangia of various sizes and shapes, as is shown in figures 60, 61, and 67. Germination of this type has not been observed very often, and so far it appears to be somewhat exceptional. In the second type an exit tube bursts through the wall as the content of the spore is transformed directly into zoospores. Figures 62–65 show surface views of such spores with the protruding exit tubes, while figure 66 illustrates a similar spore in longitudinal section. The exit canals vary considerably in diameter and length and may occasionally branch (fig. 65). Especially noteworthy in figures 62–65 are the large jagged openings made in the spore wall by the protruding tubes. The types of germination herein described are not always sharply differentiated and transition stages between the two frequently occur. Regardless of the type, posteriorly uniflagellate zoospores are eventually produced as is shown in figures 67 and 68.

NOWAKOWSKIELLA RAMOSA

This species was first observed by Butler (1907) in rotting stems of *Triticum vulgare* in India and later reported as *N. endogena* by Domjan (1936) in decaying leaves of *Typha* from Hungary. It was subsequently found in the New World by the author (1941a, 1942) in decaying vegetable debris from Texas, Alabama, Florida, Louisiana, and South Carolina. The

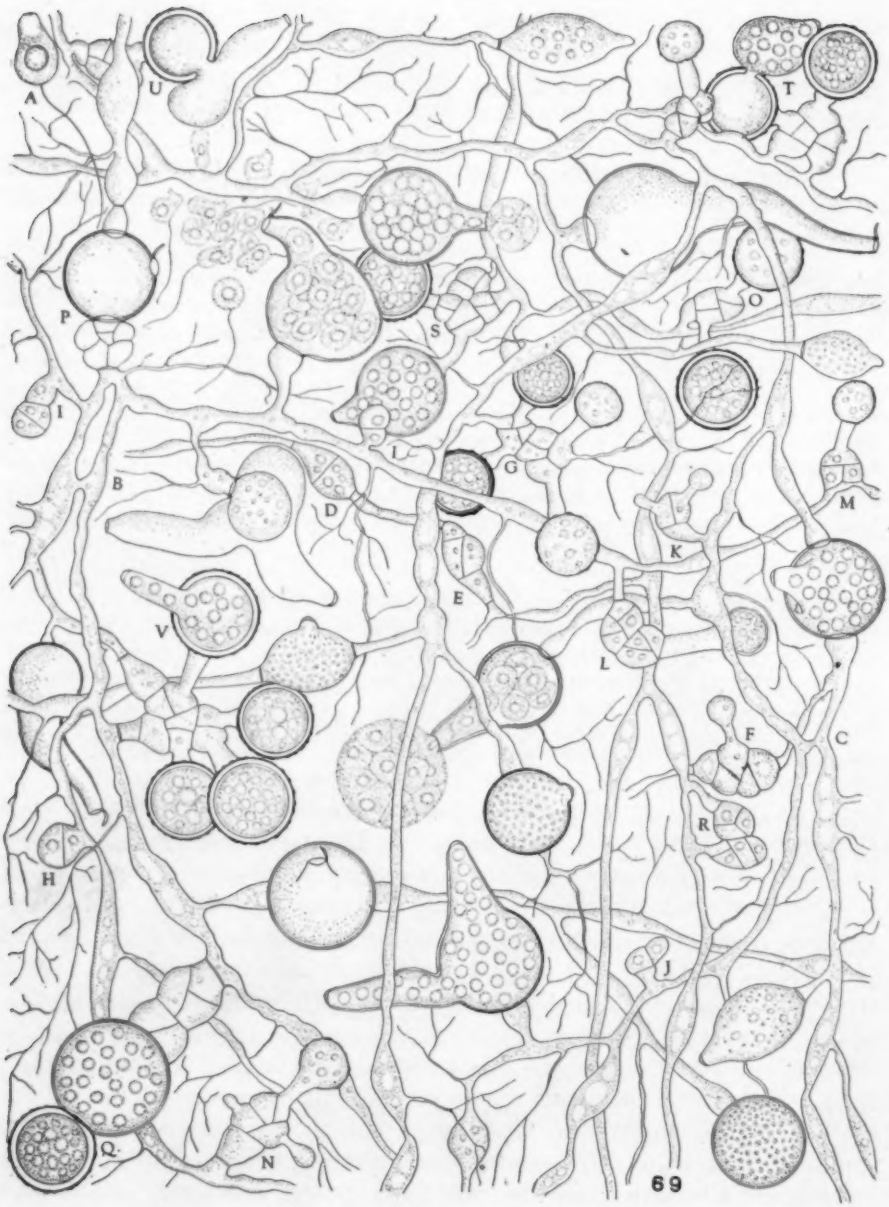
discovery of its presence in decaying grass leaves from a swamp near San Carlos, Matto Grosso, Brazil, shows that it is even more widely distributed. Study of the South and North American material has completed our knowledge of the life cycle of this species, and it is now possible to complete the species diagnosis begun by Butler in 1907.

N. RAMOSA Butler. Rhizomycelium hyaline, profuse, richly-branched, occasionally septate; tenuous portions 1.5–8 μ in diameter, occasionally anastomosing; spindle organs oval 4–6 × 6–10 μ, or broadly fusiform 5–7 × 12–16 μ, or almost spherical 6–9 μ, or elongate. Sporangia terminal or intercalary, apophysate or non-apophysate, apophysis when present usually subspherical and up to 11 μ in diameter; sporangia spherical 20–50 μ, or pyriform 15–30 × 25–40 μ, or oval 15–20 × 22–30 μ, or elongate or slightly irregular, with 1–3 low exit papillae or exit tubes up to 100 μ long. Operecula oval or circular in outline, 4–6 μ in diameter. Zoospores spherical 6.6–8.8 μ, with a large (3 μ) plastic refractive globule and a flagellum 36–40 μ long; frequently becoming amoeboid; forming a globular mass at the exit orifice immediately after emerging but soon separating and dispersing. Resting spores formed from proliferated spindle organs and short lateral branches; spherical or slightly angular, 15–26 μ, hyaline to yellowish in color, with numerous small refractive globules or a large central one surrounded by smaller globules; wall 1.8–2.6 μ thick, smooth or slightly verrucose (?); resting spores usually functioning as prosporangia in germination and giving rise to thin-walled zoosporangia on their surface; occasionally germinating directly as sporangia with an exit tube which bursts through the spore wall.

Saprophytic in decaying leaves of *Triticum vulgare* in India (Butler 1907), leaves of *Typha angustifolia* in Hungary (Domjan 1936), grass leaves and vegetable debris in the U.S.A. (Karling 1941a, 1942) and Matto Grosso, Brazil.

Nowakowskiella ramosa differs chiefly from the other known species of this genus by a marked proliferation of cells in relation to resting spore formation, and since the time of its discovery by Butler the question of whether or not sexuality is involved in this process has often been raised. For this reason particular attention has been given to this developmental phase in the Brazilian material. Inasmuch as the rhizomycelium and resting spore development of *N. ramosa* have not been extensively figured, a portion of the thallus with numerous developmental stages is presented in figure 69. When grown on cellophane the rhizomycelium may become very profuse and form a dense web of filaments, sporangia and resting spores, particularly at the edge of the substratum. Quite often almost the entire developmental cycle can be observed within a small area. Figure 69 shows a portion of such an area and illustrates very well the dense growth which this species may make.

No fundamental differences have been found between the North and South American material, except that in the latter the tenuous portions of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the thallus are often slightly coarser and more irregular. The difference, however, is not sufficiently great to warrant species differentiation. In both types of material the older filaments become fairly thick-walled with age. Hyphal anastomosis occurs fairly often (figs. 69 B, 69 C) as in other species of *Nowakowskiella*, but these fusions do not appear to have any sexual significance. At least they do not lead to the formation of zygotes.

As Butler and the author (1941a) have already reported, the resting spores of this species are formed from masses of proliferated cells in various portions of the rhizomycelium. In the Brazilian material they usually develop from spindle organs which undergo extensive proliferation. The initial stage in this process is shown in figure 69 D of an intercalary swelling which has divided transversely. Later tangential and longitudinal divisions also occur, so that the spindle organs become pseudoparenchymatous and irregular (figs. 69 E, 69 F). In the latter figure one of the cells has put forth a small stalked bud which is beginning to enlarge at the tip and will become a resting spore. A more advanced stage is shown in figure 69 G of an irregular spindle organ situated near the end of a filament and which bears two mature and two young spores. Most spores are borne on distinct stalks of varying length, and may sometimes appear in clusters up to six in number. At this point it is important to note that the development of resting spores from proliferating spindle organs is not restricted to *N. ramosa*. Similar but less extensive proliferation has been found by Berdan in *Cladochytrium hyalinum*, but in the latter species the spores apparently are not borne on stalks.

In addition to developing from spindle organs, the resting spores may also originate from short lateral branches which enlarge at their tips, divide, and undergo similar proliferation. Figures 69 H–N show various stages in this process and indicate that it is fundamentally the same as in the case of the spindle organs. Here also the spore rudiments begin as small stalked buds on the proliferating cells and develop into the mature spores (figs. 69 K, L, M). Occasionally, the proliferated lateral branches remain sterile and fail to form spores. Similar rare occurrences have been observed in the case of proliferated spindle organs also.

In this connection it may be noted that proliferation of the spindle organs and lateral branches may also be associated with the formation of

Explanation of figure 69

Nowakowskiella ramosa. A. Zoospore. × 3100. B, C. Hyphal anastomosis. × 2800. D, E, F, G. Stages in the origin of resting spores from proliferating spindle organs. × 2800. H, I, J, K, L, M, N, O. Stages in the origin of resting spores from proliferating, short lateral branches. × 2800. P. Cell proliferation in relation to sporangium development. × 2800. Q. Proliferated lateral branch, the ultimate cell of which formed a resting spore, while the penultimate cell developed into a zoosporangium. × 2800. R, S, T. Stages in the origin of resting spores from two short lateral branches which appear to have grown together and proliferated. × 2800. U. Germinated resting spores which have functioned as prosporangia. × 2800. V. Resting spore germinating directly with an exit tube. × 2800.

zoosporangia as well. Figure 69 P shows an empty sporangium which apparently developed from one of the central cells of a proliferated intercalary enlargement. Another unusual growth is shown in figure 69 Q of a greatly enlarged phragmosporous branch, the ultimate cell of which formed a resting spore while the penultimate one developed into a zoosporangium.

In a few instances resting spores were observed to arise from two lateral branches which appear to have grown together and later proliferated (figs. 69 R, S, T). In the early developmental stages such branches resemble somewhat the isogametes of a homothallic Zygomycete, and this similarity has suggested that sexuality may be involved in resting spore development. Several developmental stages of this type have been carefully followed, but so far no cell fusion between contacting branches has been observed. It is not improbable that fusions may occur, but that they involve union of gametes is not at all certain. It is quite probable that they are vegetative and of the same nature as the hyphal fusions reported above. This view of the author is supported by the fact that zoosporangia as well as resting spores may result from such union as is shown in figure 69 S.

Regardless of the type of origin of the spores, a large amount of refractive material is usually formed in the proliferating cells and eventually accumulates in the spores as they mature. The refringent globules may coalesce to form a large central one or remain relatively small and evenly distributed. The mature spore of *N. ramosa* is predominantly spherical with a fairly thick wall. A large number of spores in the Brazilian material were uneven or slightly verrucose on their outer surface, but it is not certain whether this is a true wall character or merely an incrustation. A few slightly angular spores also have been found in this material, the discovery of which supports the author's (1941b) previously expressed view that the resting spores reported by Domjan as those of *N. endogena* in Hungary actually relate to *N. ramosa*.

The resting spores germinate readily under laboratory conditions, and in so doing they usually function as prosporangia, as in most other chytrids, giving rise on their surfaces to thin-walled zoosporangia as is shown in figures 69 T and 69 V. Sometimes, however, they may germinate directly as in *N. profusa* with an exit tube which bursts through the spore wall. Figure 69 V shows such a case with a fairly long operculate exit tube.The zoospores produced by the resting spores are similar in size, shape and behavior to those formed in the primary zoosporangia.

As was noted earlier, the type species of the genus, *N. elegans*, also was found in Brazil in a moist soil sample from San Carlos, Matto Grosso. This species is widely distributed in Europe and North America, and it was not surprising to find it in South America also. Inasmuch as it is well known and has been frequently described and illustrated, further description of it here

would be superfluous, particularly since no fundamental differences were observed in the Brazilian material.

The discovery of the new fungi, *N. granulata* and *N. elongata*, raises the number of species in *Nowakowskiella* to six. Of these, all have been found in Brazil except *N. hemisphaerospora* Shanor (1942). Careful search has been made for the latter species, but so far it has not appeared in any of the soil and water samples.

SUMMARY

Five species of *Nowakowskiella* have been found in various parts of the Amazon Valley, two of which, *N. granulata* and *N. elongata*, are new. The first of these two is distinguishable by sunken opercula in the exit papillae or tubes and zoospores which contain numerous small refractive granules instead of one large, conspicuous refringent globule. The other species is characterized primarily by elongate, straight, curved, or coiled, septate or non-septate, clavate and cylindrical as well as spherical and pyriform zoosporangia. *Nowakowskiella profusa*, *N. ramosa* and *N. elegans* also occurred in great abundance in various soil and water samples collected in Brazil.

DEPARTMENT OF BOTANY, COLUMBIA UNIVERSITY
NEW YORK, NEW YORK

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INTERESTING NORTH AMERICAN AGARICS

ALEXANDER H. SMITH

INTRODUCTION

During the summer and fall of 1941, with the aid of a grant from the Horace H. Rackham School of Graduate Studies of the University of Michigan, investigations of our western agaric flora were continued in four selected localities, the Payette Lakes region of west central Idaho, the Mt. Baker region in the northern Cascades of Washington, the Olympic Mountains of western Washington, and the McKenzie Pass region in central Oregon. The last half of July and first week in August were spent in the vicinity of Payette Lakes. Here, because of favorable conditions for the fruiting of fleshy fungi, intensive collecting was carried on in the ponderosa pine forests in the immediate vicinity of the lakes as well as in the balsam and spruce forests at higher elevations to the east and southeast. During August a location was selected a short distance above Glacier on the Nooksak River near Mt. Baker and trips were made into the mountains on either side of the river. During the early part of September, in company with E. B. Mains, a survey of the Baker River area was made from a location on Baker Lake. During the last of September and most of October intensive collecting was carried on in the northern Olympic Mountains of Washington, particularly in the vicinity of Olympic Hot Springs in the Olympic National Park, and on the slopes of Mt. Angeles near the town of Port Angeles. A final location was selected during early November at Sisters, Oregon, just east of the Cascade Mountains near McKenzie Pass. In this region the open forest of pine and larch furnished excellent collecting.

Because of exceptionally favorable weather conditions, good collecting was encountered at all of these stations, and many unusual fungi were found. Since the region around Payette Lakes in Idaho is ordinarily very dry during July and early August, it was an exceptional bit of good fortune to encounter both favorable collecting conditions and a luxuriant agaric flora at that time of year. The material gathered there becomes more interesting when one recalls that almost no information on the agaric flora of the Salmon and Payette River drainage is available. The comparison of this flora with that from similar plant habitats west of the Cascades is in progress.

The Olympic agaric flora of 1941 was outstanding particularly for the large number of *Cortinarii* it contained, and critical studies of many of these will be reported upon separately. Species of *Tricholoma* and *Clitocybe* were

also unusually abundant. The same relationships noted previously (Smith 1941) between the fungous and phanerogamic floras were verified during the fall of 1941. However, certain additional features were evident. The height of the season for *Cortinarius*, particularly those species not having viscid pilei, apparently follows the fruiting period of *Boletus Lakei*, *Gomphidius oregonensis* and the varieties of *Inocybe geophylla*. Species of *Clitocybe* appear to be equally abundant in the river valleys and along the ridges although a different series occurs in each of these habitats. One interesting feature of the fruiting pattern in 1941 was the superabundance of agarics in certain restricted localities, and the time of maximum fruiting in each locality. At the time *Cortinarii* reached the peak of their fruiting cycle on Mt. Angeles and in the Olympic Hot Springs district, there were very few agarics present in the Sol Duc River Valley only a few miles to the west. In the region around Olympic Hot Springs the most abundant fruiting occurred along the ridges and steep mountain slopes just east of the Hot Springs themselves. On previous expeditions, agarics were most abundant to the west about a half mile or more up Boulder Creek. In the alder and cedar flats in the river valleys below 1500 ft. elevation the peak of the fruiting cycle preceded that of the upper Canadian and Hudsonian life zones by about two weeks. *Russula*, *Cortinarius*, *Tricholoma*, *Clitocybe*, and *Mycena* were the characteristic genera in the latter regions and *Gomphidius*, *Clitocybe*, *Lepiota*, and *Rhodophyllus* were dominant in the former, although *Russula* and *Mycena* were also well represented.

The collecting in the Mt. Baker area of northwestern Washington was not as diversified as in the Olympic Peninsula, but this may have been an apparent rather than a real difference. Because of previous expeditions into the northern Olympics, little time was wasted there searching for favorable localities, and it was possible, because of the presence of an adequate system of roads, to reach all important habitats by auto and a few hours hiking. The opposite was true in the Mt. Baker region. Much more time had to be spent on the trail, with the result that survey work progressed slowly and a more limited number of habitats could be visited. The almost continuous rain added materially to the difficulty of collecting at elevations of 2500 ft. or more, and specimens carried for long distances were often damaged. As a result, relatively few collections were obtained here in comparison with the Olympics, but many interesting agarics were found and the collecting could not be classed as poor. *Russula* was the dominant genus at all elevations, and two species of *Mycena*, *M. atroalboides* and *M. clavicularis*, were the most abundant agarics. By the time we became established at at Sisters, Oregon, the agaric season was fairly well along throughout the region west of the Cascades, but was about at its peak in the open pine country on their eastern slopes. Here an as yet undescribed *Mycena* liter-

ally covered the needles under *Pinus ponderosa*, and both *M. elegantula* and *M. laevigata* were regularly encountered on larch and pine logs and stumps. Species of *Clitocybe* were also encountered everywhere. In the forests at elevations of over 4000 ft. the agarics had already been badly frozen, and no study was possible. However, the conifer forests above this elevation in the vicinity of McKenzie Pass and Hogg Pass appear to be excellent agaric habitats in spite of the thin layer of needles and humus over the lava rock.

In the following account critical studies are presented for a few of the more interesting species collected during 1941 and a few additional agarics collected in other regions or at other times have been added. Unless otherwise stated the collection numbers are the writer's and the names of the colors within quotation marks are taken from R. Ridgway, *Color Standards and Color Nomenclature*, Washington, D. C. The names of colors not within quotation marks are used in accordance with the Ridgway system, but were not actually matched. This applies in particular to such common terms as tawny, ochraceous, avellaneous, and fuscous. The collections have all been deposited in the Herbarium of the University of Michigan.

THE GENUS AGARICUS

In a previous paper (Smith 1940) dealing with type specimens of American species of *Agaricus*, a number of Murrill's western species were omitted because the types had not been located. Since then a number of these have been found and studied with the result that certain rearrangements are necessary.

AGARICUS ABRAMSII Murrill, Mycologia **4**: 298. 1912. The type is in very poor condition, but apparently represents a young specimen of a rather robust species. Its spores measure 5–6 × 3–3.5 μ and are identical with those of the species in the *A. silvaticus* complex in both size and shape. However, most of those found on the type were immature, as was evident from their pale color. The above measurements were made on the darkest spores present. No other distinctive features such as cheilocystidia or clamp connections were found. The eccentric stipe as given in the original description was very likely just an accident of growth. This species should be excluded from the genus because it is obviously based upon an abnormal specimen.

AGARICUS SUBRUFESCENTOIDES Murrill, Mycologia **4**: 299. 1912. The type consists of an excellently preserved specimen. The pileus is covered with fine fibrillose scales, there are no remains of a fibrillose sheath below the annulus, and the spores measure 5–6.3 × 3.3–3.8 μ. The carpophore appears to be typical of *A. silvaticus* in all respects. In my own collections of the latter there are some with less and some with more pronounced scales on the pileus.

The simple annulus, as described by Murrill, must be disregarded since that of the type is typically double. My first impression from reading the description of *A. subrufescentoides* was that it might be the same as *A. subrutilescens* (Kauff.) Hotson and Stuntz. However, the color of the pileus and the lack of any signs of a fibrillose sheath below the annulus rule out this possibilty.

AGARICUS HONDENSIS Murrill, Mycologia **4**: 296. 1912. (*A. bivelatoides* Murrill., Mycologia **4**: 297. 1912. *A. Hillii* Murrill Mycologia **4**: 298. 1912. *A. McMurphyi* Murrill, Mycologia **4**: 299. 1912. *A. glaber* Zeller, Mycologia **30**: 469. 1938.)

Photographs of this species were published in a previous paper (Smith 1940) under the name of *A. glaber*, and abundant fresh material has been studied. The study of the type specimens has been greatly facilitated by the information thus gained. The species is not a particularly variable one, but does change color markedly from youth to age, and the annulus is variable insofar as the scales on the under side are concerned. The spores of *A. hondensis* (type) measure 5.5–6.5 (7) × 3.5–4 (4.5) μ. The large spores are unusual, the majority measuring 5.5–6 × 3.5 μ. The bulbous stipe and the double edged annulus are typical of the *A. silvaticus* complex. The pileus although now slightly moldy, apparently was smooth when fresh.

The type of *A. bivelatoides* has spores 5–6 × 3–3.5 μ. The annulus has the typical double edge of the *A. silvaticus* series of species even though it was described as simple by Murrill. The pilei are identical in color and surface characters with young pilei of my own collections identified as *A. glaber*.

In the type of *A. Hillii* part of the stipe is missing. However, the fungus clearly belongs in the same group as *A. silvaticus* and lacks any characters by which to distinguish it from *A. hondensis*. Its spores measure 5–5.5 × 3–3.5 μ, and the pileus is typical of a mature *A. hondensis*. Here again the "simple" annulus as described is best disregarded. Murrill did not see fresh material.

A. McMurphyi was also described from dried material. The type is a fine, large, mature and hence dark-colored specimen. The spores, double-edged annulus and bulbous stipe are all characteristic. The stipe was apparently about 2 cm. in diameter. The spores are 5–6 × 3–3.5 μ. There are no characters by which to distinguish it from *A. hondensis*.

Material of *A. glaber* from Zeller has also been examined. His specimens were collected by Dr. Burlingham under live oaks at Pacific Grove, California, and his description is the first, incidentally, to correctly characterize the annulus. All of these species have very scattered to clustered saccate cheilocystidia that are often difficult to locate because of their sporadic occurrence and because they frequently do not project beyond the basidia.

AGARICUS FLAVITINGENS Murrill, Mycologia **4**: 298. 1919. Pileus 4–10 cm. broad, broadly convex or with a flattened apex when young, expanding to nearly plane or plane with a decurved margin, nearly always having a broad flat disc in age, surface dry, shaggy-fibrillose over all except the disc and there merely matted-fibrillose, fibrils "chamois" (pale yellow) or a sordid yellow, "clay-color" (sordid yellowish brown) over the center, toward the margin the fibrils becoming arranged into appressed or slightly recurved sordid yellowish scales, white flesh showing between the scales, in age sometimes glabrescent (particularly after heavy rains) and then the entire surface more or less sordid pinkish; flesh white but quickly changing to "vinaceous pink" when cut or bruised, odor none, taste slightly nutty; lamellae "vinaceous pink" at first, becoming "Japan-rose" and finally dark sordid purplish brown, close but not crowded, almost reaching the stipe, broadest near the margin of the cap (5–7 mm.) and hence rather narrow, edges even but flocculose under a lens from cheilocystidia; stipe 4–7 cm. long, 8–12 mm. thick at apex, stuffed with a silky pith, becoming hollow, base enlarged somewhat or clavate and up to 2 cm. thick, densely white-fibrillose below the superior ring, often with fibrillose zones or patches, glabrescent, glabrous and silky above the ring, becoming sordid pink over all in age; annulus thin, submembranous to almost fibrillose, with inconspicuous yellow patches on under side in some (appearing single in many with the under side merely coarsely fibrillose with white or yellowish fibrils), glabrous and pallid on upper surface.

Spores 4–5 × 3.5–4 μ, subglobose to ellipsoid, dark fuscous in mass and under the microscope; cheilocystidia abundant, cylindric to saccate, 18–32 × 9–15 μ, thin-walled and readily collapsing.

Gregarious to subcespitose on sandy rocky soil under maple, alder, western red cedar and Douglas fir, Baker Lake, Wash., Sept. 5, 1941, *E. B. Mains & A. H. Smith 16244.*

Observations: The type of *A. flavitingens* could not be located but a water-color painting was found. It shows a fungus essentially like the above material. The yellow, fibrillose scales of the pileus and the darker brown disc, stature, and small spores appear to be distinctive of Murrill's species. Murrill described the gill color as avellaneous to umbrinous, the stipe as ochraceous tinted over the lower portion, and made no mention of a color change when the flesh was bruised. Although he described *A. flavitingens* as gregarious, his description reads as though he had drawn it from a single carpophore, and so it is very probable that no idea of the variation of the species is contained in his description. The discrepancies pointed out above should receive some consideration. The change in color when bruised, the difference in the color of the gills of young specimens, and lack of yellow tints in the stipe in age if clearly established might be significant. However, in this instance, when the inaccuracies of Murrill's descriptions are considered on the one hand, and on the other the variation established for some species of *Agaricus* such as *A. placomyces*, it appears best to disregard them at least for the time being. *A. flavitingens* appears to be closely related to *A. Kauffmani* but differs in having a fibrillose sheath below the annulus. Since

this becomes worn away in age, Murrill's description of the stipe as smooth is not a serious discrepancy in view of the fact that he probably based his description on an old specimen. In drying, the stipes of the Baker Lake collection became yellowish. Consequently this change could also be expected on old individuals which were still in the fresh condition.

THE GENUS COLLYBIA

Two previously undescribed species are included here as well as critical accounts of two that needed clarification as far as our North American flora is concerned. No attempt has been made to treat the gray, more or less hygrophanous species now placed in the genus *Lyophyllum*, although several in this group are very abundant in the western United States.

Collybia bakerensis A. H. Smith, sp. nov. Pileus convexus, glaber, pallidus vel incarnato-tinctus; lamellae angustae, confertae, breve decurrentes, albidae, saepe vinaceo-tinctae, demum flavo-maculatae; stipes crassus, aequalis, cavus, sursum albidus, deorsum subvinaceus, fibrillosus dein glaber; sporae 4.5–5.5 × 3–3.5 μ.

Pileus 2–4 cm. broad, broadly convex to obtuse when young, the margin curved in against the gills at first, expanded in age and then with or without a low umbo, in some the disc plane and the margin elevated, surface glabrous and moist, appearing appressed fibrillose under a lens in faded condition, sordid watery white when moist and some with a faint flush of incarnate, subshining when faded, opaque at all times; flesh 3–4 mm. thick over the disc and tapering gradually to the margin, watery white, odor and taste mild; lamellae very narrow (1–1.5 mm.), very crowded (58–65 reach the stipe, 4–5 tiers of lamellulae), short-decurrent to broadly adnate, white or with a faint vinaceous flush, occasionally stained sordid yellowish where bruised, edges slightly eroded; stipe short and curved, 1–3 (4) cm. long, (2) 3–5 mm. thick, equal or slightly enlarged at the base, hollow, very pliant, white above, sordid pale vinaceous brown near the base, at first covered by a thin coating of white appressed fibrils, glabrescent, inserted onto the substratum.

Spores 4.5–5.5 × 3–3.5 μ, hyaline, smooth, broadly ellipsoid, not amyloid; basidia four-spored, 18–22 × 6–7 μ; pleurocystidia not differentiated; cheilocystidia fasciculate to scattered, 24–36 × 6–10 μ, clavate, saccate or subcylindric, thin-walled and somewhat contorted or branched in age, hyaline and readily collapsing; gill trama subparallel, the subhymenium thin and ramose; pileus trama homogeneous, the surface hyphae more or less radially arranged and more compact than in the remainder, clamp connections abundant.

Singly on a conifer log, Anderson Creek, Baker National Forest, Wash., Aug. 19, 1941 (*A. H. Smith 16278*, TYPE).

Observations: When more is known of this species it may be found to be referable to *Collybia xylophila* as a variety. However it appears to differ markedly in both habitat and stature. Taken together these differences appear to be significant, and in view of the lack of information on the microscopic characters of *C. xylophila* it appears best to name the Anderson Creek

collection. The broad cheilocystidia and yellowish stains on the gills indicate that the collection is not just a depauperate form of *C. maculata* even though it is obviously closely related.

Collybia subsulcatipes A. H. Smith, sp. nov. Pileus subconicus demum planus, umbrino-vinaceus demum pallide vinaceus; odor aromaticus, graveolens; sapor mitis; lamellae confertae vel subdistantes (46–54 adnatae), latae, pallide griseo-vinaceae, crassiusculae; stipes crassus, deorsum attenuatus, radicatus, sursum pallide vinaceus, deorsum umbrino-vinaceus; sporae globosae, 5–5.5 × 4.5–5 μ.

Pileus 5.5–8 cm. broad, obtuse with an inrolled margin, becoming gibbous or plane, in age the margin recurved or elevated and frequently splitting, surface moist and polished, margin transluscent striate, hygrophanous and opaque when faded, when young "army-brown" to "vinaceous fawn" over all, disc becoming "russet-vinaceous" and margin "deep brownish vinaceous" (dark to pale vinaceous brown), fading to a "pale vinaceous buff" (pallid vinaceous); flesh thin (2–3 mm.), equal, firm and cartilaginous, concolorous with surface of pileus, odor faint but heavy and aromatic (somewhat like that of benzaldehyde) very distinctive; taste mild; lamellae close to nearly subdistant, 46–54 reach the stipe, 1–3 tiers of lamellulae, the lamellulae quite irregular in arrangement, broad (1 cm. ±), becoming slightly ventricose, depressed adnate to nearly free, color "pale grayish vinaceous" becoming "light russet-vinaceous" (gray with a tinge of vinaceous when young, becoming distinctly dull vinaceous in age), faces glaucous, edges thickish and even; stipe 6–10 (15) cm. long, 10–16 mm. thick at apex, tapered downward to a long pseudorhiza, solid and fibrous within, apex more or less concolorous with the pileus, becoming very dark sordid vinaceous brown from the base upward in age, surface pruinose but soon polished, smooth or longitudinally grooved to subsulcate.

Spores globose to subglobose, hyaline, 5–5.5 × 4.5–5 μ, smooth, not amyloid; basidia four-spored, 28–34 × 4.5–6 μ long, slender and subclavate, pleuro- and cheilocystidia not differentiated, gill trama parallel to subparallel, the hyphae more or less cylindric; pileus trama with a thin pellicle of radially arranged subgelationous hyphae 3–4 μ in dia., remainder floccose, the hyphae 5–15 μ in dia.

Gregarious on humus, Storm King Mt., Olympic National Park, Wash., Oct. 5, 1941 (*Helen V. & A. H. Smith 17566*, TYPE); same locality again on Oct. 16 (*17962*).

Observations: This species is closely related to *Collybia fusipes* but distinct because of its globose spores, odor, and vinaceous gills. *C. fusipes* has slender cheilocystidia in contrast to the absence of cheilocystidia in *C. subsulcatipes,* but no great amount of emphasis should be placed on this difference until old material of the latter can be examined. In species with filamentous cheilocystidia, these organs are sometimes slow in elongating. Excellent dried specimens of *C. fusipes* from Marcel Josserand, Lyon, France, have been compared. The most obvious difference macroscopically is in the color of the lamellae and their spacing. In *C. subsulcatipes* they are closer and more vinaceous. *C. lancipes* Fr. sensu Rea appears, from the

description, to be quite similar. It has pale flesh-color and rather thick gills as well as practically the same type of stipe. However, its spores are described as 6×4 μ, no mention is made of a peculiar odor, and the color of the pileus is given as pale reddish brown. *C. oregonensis* has a similar odor but is readily distinguished by its ellipsoid spores, the color of the gills and stipe, and in general appearance. *C. oregonensis* is closely related to *C. maculata*. *C. collybiiformis* (Murrill) Singer has some of the characters of *C. subsulcatipes* but no mention is made of a peculiar odor and the stipe and gills are described as white.

COLLYBIA CYLINDROSPORA Kauff. Pap. Mich. Acad. **5**: 126. 1926. Pileus 3–7 cm. broad, plane with a recurved and wavy striatulate margin, often lobed in age, surface glabrous and moist, "clay-color" to "pinkish buff," fading as if hygrophanous after heavy rain, becoming pallid to nearly "pale ochraceous buff" (yellowish) and retaining this color when dried or else becoming tinged alutaceous, unpolished when faded or dried; flesh thin, pliant, reviving somewhat (1.5–2 mm. thick), equal, concolorous with the surface, odor and taste not distinctive; lamellae subdistant, 28–30 reach the stipe, 3–4 tiers of lamellulae (outer two tiers very short), moderately broad (5 mm. ± near the stipe and narrowed toward the cap-margin), bluntly adnate or decurrent by a tooth, seceding, "tilleul-buff" (pallid) when young and old, drying concolorous with the pileus or slightly paler, occasionally stained with rusty brown spots, edges even but wavy; stipe 5–7 cm. long, 3–7 mm. thick at the apex, hollow, terete or compressed, very tough and pliant, velvety to unpolished and distinctly longitudinally grooved, dark brownish moist but fading like the pileus and nearly concolorous with it when dried, base strigose and with numerous white rhizomorphs.

Spores $5–6 \times 2.5$ μ, narrowly ellipsoid, smooth, not amyloid; basidia four-spored, $20–24 \times 3.5–5$ μ, subclavate; pleurocystidia not differentiated; cheilocystidia scattered to rare, filamentose, $18–30 \times 2.5–3$ μ, flexuous; gill trama interwoven, nearly hyaline in iodine (basidia brownish), subhymenium thin; pileus trama homogeneous, the surface hyphae compactly interwoven and slightly narrow than the remainder.

Gregarious to scattered under alder, Park Creek, Mt. Baker, Wash., Sept. 10, 1941 (*16811*); Baker Lake, Wash., Sept. 13 (*16936*); Chimacum, Wash. (Olympic Peninsula), Oct. 13, 1941 (*17856*).

Observations: C. cylindrospora is closely related to *C. confluens* and *C. hariolorum*, but is readily distinguished by its sulcate striate or channeled stipe and broad subdistant gills. Among Kauffman's collections two of this species were found. One, the type, and a second identified as *C. prolixa* Fr.—Ricken. Apparently this second collection is the basis for Kauffman's report of *C. prolixa* from Mt. Hood. The only other collection of *C. prolixa* located was an earlier collection which he made at Lake Cushman, Washington, in 1915. This one is much closer to the European concept and is the same species Murrill described as *C. badiialba*. For an account of the latter see Smith (1941). Regardless of whether or not *C. prolixa* and *C. badiialba* are synonymous, the report of the former from Mt. Hood cannot

be recognized. Although Kauffman compared his new species with *C. fusipes*, *C. lancipes*, and *C. distorta*, actually there is little or no resemblance to them. *C. cylindrospora* is a *Marasmius*-like fungus with the relationships pointed out above. An examination of Kauffman's type shows that the stipes were unpolished to velvety or at the most glabrous above only. The spacing of the gills appears to be variable, but in all the dried material studied they were most accurately characterized as subdistant. My description was drawn from the material cited above, and is given to supplement that of Kauffman.

The dominant color of the pileus is whitish-alutaceous to yellowish and when properly dried whitish buff with an unpolished appearance. Young or water-soaked specimens have the dark colors given in the description. In general the colors appear to be similar to and to vary much the same as those of *Marasmius oreades* (whitish to rather dark alutaceous), and do not appear taxonomically significant within these limits. The broad subdistant gills and the channelled velvety to unpolished dark colored stipe are the important characters aside from the spores.

More than likely *C. cylindrospora* is a synonym of *Collybia laxipes* (Fr.) Quél., but more information on the microscopic characters of the latter is needed to be certain. Rea (1922) gives the spores of the latter as $5–6 \times 3\ \mu$ and elliptical. This may indicate a difference in shape. Rea's description of *C. laxipes* covers the diagnostic features of *C. cylindrospora* remarkably well, and his account is closely in line with the Friesian concept. Quélet's (1873) illustration, however, depicts very small atypical individuals if they are to be judged on the basis of the descriptions of most authors and the Washington and Oregon collections here placed in *C. cylindrospora*.

COLLYBIA EXTUBERANS (Fries) Quélet, Champ. Jura & Vosges, 97. 1872. Pileus 2–5 cm. broad, conic with an inrolled margin when young, becoming broadly conic-campanulate or plane with a low or prominent conic umbo, glabrous, surface viscid when wet but soon dry and very finely radially wrinkled, the margin very thin and soon recurved, sometimes becoming faintly translucent-striatulate, color "bone-brown" (very dark brown with a tinge of red) over all when young, gradually becoming a dull shade of "pecan-brown" or a paler vinaceous brown (but not as red as in *C. badialba*); flesh thin, pliant and moderately tough, very sordid reddish brown near the pellicle, paler below, gradually becoming pallid throughout, odor none, taste mild; lamellae close to crowded, 48–56 reach the stipe, 2–3 tiers of lamellulae, distinct, sinuate and attached only by a tooth, narrow to moderately broad (3–5 mm.) equal or narrowed from the stipe to the margin of the cap, "pale pinkish buff" or with a more vinaceous cast (pallid buff to pinkish buff), becoming spotted with very sordid brownish spots, edges only slightly eroded; stipe 4–7 cm. long, 3–6 mm. thick above, more or less tapered at the base to a pseudorhiza-like prolongation which ends in one or more rhizomorphs, longitudinally striate, glabrous, pallid (concolorous with young gills) above, becoming sordid brownish progressively downward (base almost concolorous with the pileus in age).

Spores hyaline, 4.5–6 × 3–3.5 μ, narrowly ellipsoid, smooth, not or only very weakly amyloid (reaction not conclusive); basidia four-spored, 18–26 × 5–6.5 μ, clavate; pleurocystidia and cheilocystidia not differentiated; gill trama of more or less parallel cylindric hyphae 8–15 μ in dia., subhymenium very thin and ramose; pileus trama with a pellicle of radially arranged smooth-walled hyphae 3–4 μ in dia., the layer 2–3 hyphae thick, beneath this a layer of interwoven subgelatinous hyphae with clamp connections, the hyphae 3–5 μ in dia. and the layer 15–30 μ thick, the remainder of the flesh floccose, the hyphae 5–15 μ in dia., the pigment dissolved in the cell sap and most abundant just below the subgelatinous layer.

Cespitose to gregarious on rotten conifer logs, Lake Angeles, Olympic Mts., Wash., Sept. 19, 1941 (*16967*); Mt. Angeles, Sept. 28 (*17368*); Olympic Hot Springs, Olympic National Park, Oct. 11, 1941 (*17800*).

Observations: This species is closely related to *Collybia badiialba* Murrill, but differs distinctly in the shape of its spores, in the organization of the pellicle of the pileus, and in the duller colors as well as in the less crowded gills and radicating stipe. It is also more inclined to be cespitose, but this appears to be a variable character. *C. extuberans* belongs to the same series of species as *C. fusipes*. It apparently differs from the latter in habitat, close, narrower lamellae and more than likely in the organization of the pileus. Cooke's illustration cited for *C. extuberans* by Rea does not depict the species satisfactorily. The stipe, as shown by Cooke, does not taper to rootlike projection and the umbonate character of the pileus is not sufficiently emphasized. Rea's description, however, appears to apply very well and that of Ricken is about equally good. Neither mention the reddish stains which develop on the gills. However, this character appears to be common to most of the radicating *Collybiae* of this series so I would hesitate to use its presence here as a character of any significance, particularly when *C. extuberans* is not too well known.

THE GENUS CLITOCYBE

Although one of the more uninteresting genera of the agarics, the species of *Clitocybe*, both by the abundance of fruiting bodies produced and the large number of species present in our flora, make continued demands on the time of the agaric specialist. They are a rather difficult group because of the general lack of distinctive microscopic characters and intergradation of the macroscopic characters by which species are usually distinguished. In the following account I have brought together the information accumulated on the more unusual species of this genus during the past eight years. Seven species are described as new and accounts of two others which were incompletely or erroneously described are included.

Clitocybe flavissima A. H. Smith, sp. nov. Pileus convexus demum late convexus, siccus, fibrillosus, laete luteus; sapor subpiperatus; lamellae subdistantes, latae, late adnatae, laete luteae; stipes aequalis, luteus, fibrillosus; sporae globosae vel subglobosae, 7–9 μ; cheilocystidia 40–200 × 3–5 μ; ad truncos coniferarum, solitarius vel caespitosus.

Pileus 3–5 cm. broad, convex, becoming broadly convex, surface dry and innately fibrillose or moist beneath the fibrils, either remaining fibrillose or becoming more or less scaly around the disc, the fibrils near the margin sometimes arranged into fascicles and the margin usually fimbriate when young, evenly yellow over all ("mustard-yellow") or the disc darker and near "antimony-yellow"; flesh thin but nearly equal (1.5–2 mm.), pliant, dull yellow, odor faintly fragrant, taste slightly peppery; lamellae nearly subdistant, 24–30 reach the stipe, 2 tiers of lamellulae, moderately broad (5 mm. ±), bluntly adnate but becoming more or less decurrent, bright yellow ("primuline-yellow") becoming "light orange-yellow," edges a brighter yellow than the faces and appearing gelatinous under a lens; stipe 2–5 cm. long, 3–6 mm. thick, equal, hollow, fleshy, concolorous with the pileus in age, pallid when young from the thin pale yellowish partial veil, in age appressed fibrillose to the apex.

Spores globose to subglobose, 7–9 μ, smooth, not amyloid; basidia four-spored; pleurocystidia none, cheilocystidia very abundant, 40–200 × 3–5 μ, septate, filamentose, subgelatinous, yellowish to hyaline when fresh, the pigment intracellular; gill trama homogeneous, the cuticle of radially arranged hyphae with thickened walls and abundant clamp connections.

Scattered to subcespitose on dead conifer stubs, Ermine Creek, Baker National Forest, Wash., Sept. 11, 1941 (*16837, 16928*); Mt. Angeles, Olympic Mountains, Wash., Sept. 21, 1941 (*17084,* TYPE).

Observations: C. flavissima is very closely related to *C. decora* but is readily distinguished by its globose spores, long filamentose cheilocystidia and lack of brownish scales on the pileus. These two, *Tricholoma rutilans, T. flavescens* and *Pleurotus sulfureoides* form a very natural group or stirps and make up the nucleus of the genus *Tricholomopsis* Singer. In a recent article Singer (1942) added *Tricholoma radicatum* Pk. and *T. secedifolia* Murrill to *Tricholomopsis. Collybia platyphylla* was previously placed here. I have not studied the types of *T. flavescens, P. sulfureoides,* or *Clitocybe sulphurea* Pk., but from other material believe that all are identical. The long filamentose subgelatinous cheilocystidia readily distinguish *C. flavissima* from all the species of the *Tricholomospsis* series.

Clitocybe glutiniceps A. H. Smith, sp. nov. Pileus late convexus demum planus (non depressus), viscidus, glaber, striatus, sordide cremeus; lamellae confertae, latae, arcuatae vel brevissime decurrentes, pallide sordido-cremeae; stipes elasticus, canescens, pallidus; sporae 4.5–5 × 3–3.5 μ.

Pileus 2.5–4 (6) cm. broad, broadly convex with an inrolled margin when young, becoming nearly plane (the disc not depressed), surface glabrous and viscid to the touch, the pellicle somewhat separable, translucent striate on the margin, color evenly pale dirty cream color or in age the disc tinged sordid brown; flesh thin, pliant, concolorous with surface, odor none, taste mild, no color change when bruised or cut; lamellae moderately close, 28–33 reach the stipe, 2 tiers of lamellulae, broadest (2–3 mm.) at attachment, arcuate-adnate to short decurrent, seceding, pale sordid cream color (paler than the pileus), edges even; stipe short, 3–4 cm. long, 4–6 mm. thick at the apex, hollow, rather tough and pliant, whitish from a coating of appressed fibrils which extends to the apex, base sparsely white strigose.

Spores 4.5–5 × 3–3.5 μ, ellipsoid, smooth, not or only very weakly amyloid (reaction doubtful); basidia four-spored; pleurocystidia and cheilocystidia not differentiated; gill trama of narrow interwoven non-amyloid hyphae, subhymenium subgelatinous (in KOH) when revived; pileus trama floccose beneath a somewhat gelatinous pellicle of interwoven hyphae 2–4 μ in dia., hyphae of the flesh 6–15 μ in dia.

Gregarious under conifers, Olympic Hot Springs, Olympic Mountains, Wash., Oct. 2, 1941 (*17524*, TYPE).

Observations: Although this collection was made under very adverse weather conditions, the carpophores were in good condition and the viscidity of the pileus cannot be disregarded. The pellicle is made up of very narrow hyphae in contrast to those forming the flesh, and exhibits the characters of a truly viscid layer rather than the false viscidity often encountered on various species such as *Inocybe geophylla* in this region.

Because of the somewhat cartilaginous character of the stipe some might be inclined to place the species in *Omphalia*. It is described in *Clitocybe* because of its relationship to *C. brumalis*. Lange's (1935) concept of the latter is very close, but there appears to be a distinct difference in shape of the pileus and the nature of its surface. *C. obsoleta* is also close but apparently differs in having an anise-like odor, in not possessing a viscid pellicle and in the margin of the pileus not being translucent-striate. Lange gives the spores of the latter as 6 × 3–3.5 μ, Rea as 7 × 4–5 μ, and Ricken as 6–7 × 3–4 μ. These measurements are consistently longer than those of *C. glutiniceps*, and indicate an appreciable difference in shape, i.e., ellipsoid contrasted to subglobose.

Clitocybe gomphidioides A. H. Smith, sp. nov. Pileus convexus mox subplanus vel subdepressus, viscidus, glaber, vel minute areolatus, subferruginus; sapor valde farinaceus; lamellae subdistantes vel confertae, crassae, venosae, pallidae mox sordide avellaneae; stipes solidus, pallidus vel sordide avellaneous; sporae (7) 8–10 (11) × 4–5.5 μ.

Pileus (3) 5–9 cm. broad, at first plane with an inrolled margin or very slightly arched, expanding to very broadly convex with a depressed disc or plane, in age the margin often elevated and disc broadly depressed, not truly infundibuliform, surface viscid, glabrous or with minute areolate or spotlike scales over the central portion, margin minutely tomentose, color "Mikado brown" on the disc but "orange-cinnamon," "pinkish cinnamon" or "light pinkish cinnamon" toward the margin (color reddish cinnamon on disc, paler cinnamon to ferruginous-cinnamon toward the margin), in age fading to "sayal brown" on disc, (duller and more yellowish cinnamon), the margin remaining "pinkish cinnamon," sometimes dull tan with scarcely a trace of red in age, regaining the reddish cinnamon colors when dried; flesh thick and tapered abruptly to the margin, pale "vinaceous buff" and watery punctate, odor and taste very strongly rancid-farinaceous; lamellae subdistant to close, 50–58 reach the stipe, 2 tiers lamellulae, many forked either near the stipe or part way to margin, narrow to moderately broad (3–5–8 mm.), tapered each way, short decurrent, often thickish, intervenose

or wrinkled, near "light pinkish cinnamon" young, "avellaneous" to "wood brown" in age (dark avellaneous); stipe 3–7 cm. long, 9–15 mm. thick, solid, "pale vinaceous buff" within or darker in the base, surface paler than the gills and with a thin coating of pallid appressed fibrils, glabrescent and darker in age, when dry more or less concolorous with the pileus.

Spores (7) 8–10 (11) × 4–4.5 μ, narrowly ellipsoid to subfusoid, not amyloid, smooth, white in mass; basidia four-spored; pleurocystidia and cheilocystidia not differentiated; gill trama not amyloid; pileus trama with the pellicle made up of an interwoven mass of narrow hyphae with subgelatinous walls, the hyphae 3–5 μ in dia. and not appreciably thinner than those of the flesh, clamp connections abundant.

Gregarious under Devil's club on wet soil, Olympic Hot Springs, Olympic National Park, Washington, Oct. 2 (*17504*) and Oct. 10, 1941 (*17682*, TYPE), also at Lake Mills near Wolf Creek, Oct. 12, 1941.

Observations: The outstanding characters of this species are the reddish cinnamon color, viscid cap, gills which change color in aging, long rather narrow smooth spores and very pronounced odor and taste. *Clitocybe incilis* and *C. opiaria* appear to be the most closely related species. The former is not described as viscid and is generally said to lack a farinaceous odor and taste. The illustration by Nuesch (1931) is very suggestive of *C. gomphidioides*, however. Since Fries placed *C. incilis* next to *C. sinopicus* and described the pileus as "non udus" it hardly appears justifiable to place a viscid species under the Friesian name. *C. opiaria* also differs in not having a viscid pileus, and its gills are not described as becoming dark avellaneous. The odor and taste are also different according to descriptions.

In the Olympics, where various species of *Gomphidius* are very abundant, one could easily pass by this *Clitocybe* thinking it was just an abnormal growth of a reddish *Gomphidius*. The thick, veined, forked, dark-colored gills at maturity, the stature of the carpophore, and the color of the pileus all contribute to this impression. The spores also remind one of the spores of a *Gomphidius* because of their subfusoid shape. However, they are white in mass and since other members of *Clitocybe* have similarly shaped spores, the resemblance does not appear significant.

Clitocybe michiganensis A. H. Smith, sp. nov. Pileus late convexus vel subplanus, cinereo-canescens demum politus, hygrophanus, olivaceo-fuligineus mox pallidus; lamellae confertae, adnatae vel subdecurrentes, olivaceo-fuligineae; stipes aequalis, cavus, cinereo-canescens demum glaber et fuligineus; sporae 4–4.5 × 2.5 μ.

Pileus 1–2.5 cm. broad, very broadly convex to nearly plane and with an inrolled margin at first, becoming broadly convex to plane in age, surface appearing dry at first from a hoary-canescent thin coating of fine gray fibrils, glabrescent and then moist and polished, when covered with fibrils appearing pale glaucous gray, when polished evenly "hair-brown" to "drab" (nearly putty color), hygrophanous and fading to pale gray or whitish, usually fading on disc first; flesh thin, equal, concolor with surface, watery, taste strongly rancid-farinaceous, odor faintly so; lamellae close to almost crowded, 30–40

reach the stipe, 2 tiers of lamellulae, adnate at first, short-decurrent at maturity, narrow, nearly equal, "drab" or with an olivaceous tinge when young, sometimes paler and more sordid in age; stipe 2–3 cm. long, 2.5–5 mm. thick equal or slightly enlarged below, hollow, grayish within, surface covered like the pileus with a thin coating of glaucous fibrils, glabrescent and then concolorous with the naked cap.

Spores 4–4.5 × 2.5 μ, narrowly ellipsoid, not amyloid, smooth, basidia four-spored; pleuro- and cheilocystidia not differentiated; gill trama not amyloid, of moderately broad hyphae; pileus trama homogeneous, not amyloid, clamp connections present.

Scattered or solitary on debris in oak woods, Oct. 30, 1940, Ann Arbor, Mich. (*15505*, TYPE).

Observations: When the canescent fibrillose covering of the cap and stipe have been worn away, this species is similar in color to *C. ditopoda*. It differs from the latter in its narrowly ellipsoid spores, pronounced canescent fibrillose coating over pileus and stipe when young, strongly rancid-farinaceous taste, and the much smaller size. In addition, *C. michiganensis* appears to fruit in a solitary or widely scattered manner rather than being densely gregarious as in *C. ditopoda*. *C. mortuosa* sensu Ricken differs in having paler gills and a glabrous pileus. *C. vilescens* Pk. is almost identical in appearance with *C. michiganensis* but can be distinguished by its taste and microscopically by its spores.

During the season 1937 *C. vilescens* was collected in a pasture in southern Oregon. The specimens were fruiting on the accumulated debris around an old oak stub, Kirby, Oregon, Nov. 26, 1937 (*9092*). The spores measure 5–6 × 4–4.5 μ, are smooth, broadly ellipsoid to subglobose, and not amyloid. The basidia are four-spored, no cystidia are differentiated, the gill trama is composed of narrow hyphae, and the pileus trama has a cuticle of interwoven hyphae 2–4 μ thick, the hyphae of the trama itself are 5–10 μ thick. Clamp connections are present. The macroscopic characters are as Kauffman (1918) gave them except that the margin of the pileus may be faintly striatulate in age when wet, and the gills may be rather dark cinereous.

Clitocybe piperata A. H. Smith, sp. nov. Pileus convexus vel late convexus, pruinosus et pallidus, mox politus et alutaceus; sapor valde acris; lamellae confertae vel subdistantes, adnatae vel subdecurrentes, angustae, pallidae demum olivaceo-cinereae; stipes clavatus, solidus, impolitus, sursum pruinosus, pallidus; sporae 4–5.5 (6) × 2–2.5 μ.

Pileus 6–12 cm. broad, convex to obtuse and with an inrolled margin when young, in age broadly convex to plane or the margin wavy and uplifted slightly, regular or occasionally either sinuate or lobed, surface at first moist beneath a hoary pruinose covering caused by fine projecting microscopic hairs 20–42 × 3–4 μ, becoming glabrous and occasionally with watery spots around the disc, opaque, color pallid at first ("tilleul-buff"), or very pale avellaneous with a chalky white sheen, gradually becoming dull yellowish brown ("clay-color") when still moist, fading as if hygrophanous (in old caps) to "light buff" (dull yellowish), when dried varying between

pale buff and alutaceous; flesh thick (about 1 cm. on the disc), tapering evenly to the margin, pallid to pale buff, somewhat punky in texture, odor faintly fragrant in young caps, becoming somewhat disagreeable at maturity, taste very sharply acrid (as in *Russula emetica*) and sometimes with a bitter after taste, no color change when cut or bruised. Lamellae nearly crowded in small caps, almost subdistant in large ones, rounded or bluntly adnate and becoming sinuate with a short decurrent tooth or becoming merely short-decurrent, narrow to moderately broad (5 mm. ±), "tilleul-buff" (pallid) at first, gradually becoming darker, pale olive-buff to subavellaneous ("olive-buff" to "pale olive-buff") or in age fairly dark avellaneous, "pinkish buff" (yellowish) in dried specimens, edges even to eroded and thickish in some specimens; stipe 4–9 cm. long, 1–2 cm. thick at the apex, clavate, 2–3 cm. at base, solid, usually somewhat pointed below the enlarged portion, rather tough to punky and firm in texture, pallid to pale buff within, surface unpolished at first, more or less pruinose toward the apex, base slightly cottony and with a few rhizomorphs, glabrous but somewhat scaly in age from the torn cuticle.

Spores 4–5.5 (6) × 2–2.5 μ, subcylindric to subfusiform, smooth, (a few appearing very slightly wrinkled under an oil immersion lens), not amyloid; basidia four-spored; pleuro- and cheilocystidia not differentiated; gill trama subparallel, the hyphae with long and moderately wide (5–8 μ) cells, not amyloid; pileus trama floccose beneath a thick cuticle of compactly interwoven hyphae 4–8 μ in dia. which give rise to numerous projecting hairs 3–4 μ thick, not amyloid and the walls not truly gelatinous in KOH, clamp connections rare.

Gregarious around or on old stumps and logs in low hardwood forests, Pontiac, Mich., Aug. 24, 1937 (*7212*); Kent Lake, Oakland County, Mich., Sept. 24, 1940 (*15462*, TYPE); same locality, July 31, 1942 (*18540*); Dexter, Mich., Aug. 14, 1942 (*18671*) and Aug. 17 (*18735*).

Observations: The outstanding features of this species are the small narrow spores, acrid taste, olive-buff gills in age, and the progressive color change of the pileus as it matures. *C. piperata* appears to be related to *C. innornata* by its gill characters (both attachment and color), its spores, and by the broadly convex to plane pileus. The spores vary in shape from almost truly cylindric to somewhat sway-backed or ventricose and hence are subfusoid. Such small narrow spores as this are known for several different species in *Tricholoma* and *Clitocybe*, but in no species of either genus have I found the combination of characters listed above.

Clitocybe sublutea A. H. Smith, sp. nov. Pileus convexus dein late depressus, glaber subviscidus, striatus, hygrophanus, pallide luteo-alutaceus demum pallidus; lamellae subdistantes, decurrentes, latae, pallide sulphureae; stipes aequalis, glaber, sursum pallide sulphureus, deorsum subalutaceus; sporae 4.5–5.5 × 3.5 μ.

Pileus 2.5–5 cm. broad, broadly convex with an incurved margin when young, in age the margin uplifted and the disc broadly depressed, glabrous and slightly viscid when moist, margin translucent-striate, color "cartridge-buff" over all when young (very pale yellowish), in age darker on the disc and near "pinkish buff" (buff), hygrophanous, pallid when faded; flesh

thick on the disc (4–6 mm. near the stipe), tapering rapidly to margin, concolorous with cap in either moist or faded condition, not changing when bruised, odor and taste not distinctive; lamellae subdistant, 2–4 tiers of lamellulae, long- and unequally-decurrent, broad (5–7 mm.), tapered each way, color "ivory yellow" (pale yellow) both in young and old specimens, not changing when bruised, edges wavy; stipe 5–7 cm. long, 4–7 mm. thick, usually somewhat eccentric or the cap aborted on one side, hollow, equal or narrowed downward, glabrous above, concolorous with the gills near apex, darker downward, cinnamon-buff at the base in age, base sparsely white strigose.

Spores 4.5–5.5 × 3.5 μ, ellipsoid, smooth, not amyloid; basidia four-spored; pleuro- and cheilocystidia not differentiated; gill trama somewhat interwoven, not amyloid, the hyphae narrow; pileus trama homogeneous, the hyphae on the surface the same size or slightly smaller than the others and distinctly gelatinous, clamp connections abundant.

Gregarious under alder, Port Angeles, Wash., Oct. 13, 1941 (*17830*, TYPE).

Observations: The pale yellowish colors, subdistant gills, very small ellipsoid spores, and lack of a farinaceous or bitter taste distinguish this species. I hesitate to place much emphasis on the viscidity of the pileus because the pilei had been subjected to steady rain during most of their development. The species appears to be most closely related to *C. subinversa*, but the spores definitely distinguish it. It differs, apparently, from *C. obsoleta* in its yellowish subdistant gills as well as in the more pronounced yellowish color of both cap and stipe. From *C. subalutacea* sensu Ricken it differs in having an hygrophanous pileus and larger ellipsoid spores. In stature *C. sublutea* bears a striking similarity to *C. ectypa* sensu Lange and other recent European authors. However, it differs in the lack of fuscous fibrils over the disc and in having smaller spores. *C. hypnorum* (Brond.) Rea is very similar in many respects but is said not to be hygrophanous and to have "somewhat crowded" gills. The American species should also differ in having a striate, subviscid pileus. *C. isabella* Quélet is also a somewhat similarly colored species, but from available information does not appear to be identical.

Clitocybe virgata A. H. Smith, sp. nov. Pileus convexus vel planus, sinuosus, siccus, fibrillosus demum subsquamulosus, disco cinereo-fuscus, ad marginem pallidus; lamellae angustae, confertae, adnatae vel brivissime decurrentes, albidae demum cremeae; stipes clavatus, deorsum 3 cm. crassus, solidus, albidus, sursum subpruinosus, deorsum impolitus, glaber; sporae 5–6 μ, globosae.

Pileus 5–14 cm. broad, broadly convex or flattened and the margin decurved at first, more or less expanded to plane in age or margin undulating or wavy and more or less uplifted, often sinuate or irregular, surface appressed fibrillose, dry, color dirty grayish brown over the center and streaked grayish on a pallid ground color toward the margin, in age becoming minutely fibrillose-scurfy, margin pallid to sordid buff; flesh thick, firm, dry, white, unchanging or slowly pale buff where bruised or cut, odor and

taste not distinctive; lamellae narrow (5–6 mm.), close, adnate when young but short decurrent at maturity, many tiers of lamellulae, whitish but becoming cream-colored in age or stained brownish where damaged by insects, intervenose in small caps, edges even or eroded; stipe 5–10 cm. long, 1–2 cm. thick at apex, up to 3 cm. at base, clavate, solid, white within, surface white and unchanging, fibrillose-pruinose near the apex, glabrous below but dull and unpolished, base white cottony.

Spores globose, 5–6 μ, smooth, not amyloid; basidia four-spored; pleurocystidia and cheilocystidia not differentiated; gill trama subregular, not amyloid; pileus trama not amyloid, homogeneous beneath a compact pellicle of interwoven hyphae about the same size as those of the flesh, clamp connections present but rare.

Gregarious in an oak woods on humus, Oakland County, Mich., Sept. 24, 1940 (*15468*, TYPE).

Observations: The grayish streaked pileus reminds one of that of *Collybia platyphylla*, but the resemblance is superficial. The closer, short-decurrent gills, globose spores and lack of cheilocystidia distinguish it at once. It appears to be most closely related to *Clitocybe nebularis* from which its spores readily distinguish it. The description of *Clitocybe fumosa* in the North American Flora reads a good deal like the above, but the cap is described as smooth and glabrous. European authors are generally agreed that *Agaricus fumosus* Pers. is a member of the series of forms grouped around *C. aggregata*. These are variously placed in *Tricholoma*, *Clitocybe*, and *Collybia*, and are not truly related to the fungus described here. Most of them probably belong in the modern genus *Lyophyllum*. It is possible that *Tricholoma tenuiceps* Cke. & Massee is identical with *C. virgata*, but with the information available at present this does not seem likely. *T. tenuiceps* may also be a member of the *C. aggregata* complex, and, to judge from the description, its pileus does not become fibrillose-squammulose. Von Höhnel (1919) transferred *T. tenuiceps* to *Russula* but his study was not based on authentic specimens. Pearson (1935) lists the opinions of Quélet, Maire, and Rea on Cooke's plate 1166. Maire was the only one to suggest a similarity to *Collybia platyphylla*, and he made that questionable. From Cooke's illustration it is apparent that the gill attachment in *T. tenuiceps* is quite different from that of *C. virgata*.

CLITOCYBE ALEXANDRI (Gillet) Konrad, Bull. Soc. Myc. Fr. **43**: 186. 1927.
?*Clitocybe Harperi* Murrill, Mycologia **3**: 190. 1911.

Pileus 6–15 cm. broad, plane with a strongly inrolled margin at first, soon shallowly depressed, in age the margin elevated and frequently lacerated or irregular giving the caps a broadly infundibuliform appearance, the margin long remaining inrolled, surface smooth and glabrous at first but appressed felty-fibrillose if viewed under a lens, disc becoming more or less fibrillose-furfuraceous, typically dry (but when water-soaked appearing subhygrophanous) occasionally mottled with large watery spots, color sordid and near "vinaceous buff" at first, more alutaceous when moist or water-soaked (but

such caps fading to "tilleul-buff," hence pallid), in aging gradually becoming grayish, old caps sometimes sordid drab; flesh thick, ± 15 mm. near the stipe, tapered evenly to the margin, pallid but gradually becoming grayer in age, odor and taste not distinctive; lamellae crowded, narrow (4–5 mm.), 3–4 tiers of lamellulae, adnate but soon becoming decurrent, somewhat intervenose, pallid when young ("tilleul-buff") but gradually cinerescent and in age pale dirty gray to dark sordid brownish, separable, edges even; stipe 5–10 cm. long, 10–30 mm. thick, clavate, solid, pallid within, surface uneven, unpolished to appressed fibrillose, concolorous with pileus and discoloring in the same manner, becoming quite sordid in age.

Spores 4–4.5 × 3–3.5 μ, smooth, broad, white in mass, not amyloid; basidia four-spored; pleuro- and cheilocystidia none seen; gill trama homogeneous, not amyloid, the hyphae somewhat interwoven; pileus trama homogenous, the cuticle of narrow interwoven hyphae, not amyloid.

Scattered under spruce, Fort Dick, Calif., Nov. 10, 1937 (*8602*); Crescent City, Calif., Dec. 7, 1937 (*9490*); under alder and cedar, Port Angeles, Wash., Oct. 13, 1941 (*17824*).

Observations: This species is not uncommon along the Pacific Coast from Washington to California. It can readily be distinguished from *Clitocybe nebularis* by its changing gills and generally more sordid appearance. In addition its spores are consistently a little smaller. Lange's (1935) illustrations depict our West Coast form more accurately than do those of other authors. Partly developed pilei are likely to be squatty in appearance and to have very thick stipes. This is the stage figured by Konrad and Maublanc (1924–1933). This species, to judge by the few references to it in the literature, is either rare or rarely collected and identified. Since it is so unattractive, it is possible that many collectors have passed it by thinking that the material was too old to study. It apparently has not been reported from North America, but an examination of the descriptions of new species of West Coast *Clitocybeae* published by Murrill reveals that *C. Harperi* is very likely identical. Singer (1942) has reported a study of the type of the latter in which he pointed out that the species is closely related to *C. nebularis*. This in addition to the cinereous gills of *C. Harperi* certainly indicates identity with *C. Alexandri*. However, I have not had an opportunity to compare my specimens with the type. Konrad (1936) has discussed at some length the synonymy of this species, and has pointed out that *Paxillus griseo-tomentosus* (Secr.) is very likely identical and that the combination *Clitocybe griseotomentosus* (Secr.) Konrad ought to be used if the synonymy is accepted and the rules regarding priority are rigidly adhered to. Like Konrad, I have used the name Gillet gave to the species in order to be consistent with recently established usage and to avoid accepting a name that has long been doubtful and over which there might easily be a difference of opinion in the future.

CLITOCYBE AVELLANEIALBA Murrill, Mycologia **5**: 207. 1913. Pileus (4) 8–12 (20) cm. broad, when young obtusely umbonate with a flattened mar-

ginal area and an inrolled margin, sometimes more or less convex, becoming plane or slightly depressed, the umbo sometimes obsolete, in age at times broadly infundibuliform, margin minutely grayish-fibrillose-pubescent, surface moist, slightly scabrous in some, cuticle occasionally broken into scales in age, toward the margin somewhat fibrillose-streaked, not truly hygrophanous (when water soaked appearing subhygrophanous), color "clove-brown" to "mummy-brown" when young and moist (nearly black with very little brown), fading slowly to "drab" and finally dark or pale cinereous to avellaneous, margin ribbed or plicate in some; flesh white, not watery, thin, pliant, odor and taste slightly pungent and subrancid; lamellae narrow (4–5 mm.) nearly equal in width and unequally decurrent on the stipe, close but not crowded, 57–64 decurrent on the stipe, 2 tiers of lamellulae, pallid at first ("tilleul-buff"), in age becoming slightly cream colored but the change often not pronounced, edges even; stipe 6–12 (18) cm. long, 1–3 cm. thick at apex, clavate, up to 4 cm. thick at base in large carpophores, stuffed becoming hollow, cortex thin but tough, surface glabrous and uneven, unpolished at first but in age more or less polished, sometimes with scattered appressed fibrils, concolorous with or paler than the pileus, base with only a thin appressed white mycelium and either abruptly rounded below or drawn out to a point causing the stipe to appear subfusiform.

Spores 8–10 (11) × 4–5.5 μ, broadly fusiform or somewhat inequilateral, hyaline, smooth, not amyloid; basidia four-spored; pleuro- and cheilocystidia not differentiated; gill trama not amyloid, of interwoven hyphae; pileus trama characterized by a thin pellicle of slightly gelatinous hyphae (in KOH) with dark brown contents, the remainder floccose and hyaline, not amyloid, clamp connections abundant.

Gregarious to subcespitose on humus and rotting wood, under alder or mixed conifers, common. Crescent Beach, Joyce, Wash., Oct. 3, 1935 (*2846*) and Sept. 24 (*2582*); Orick, Calif., Dec. 5, 1935 (*3790*); Trinidad, Calif., Dec. 11, 1935 (*3938*); Martin Lake Trail, Mt. Baker, Wash., Sept. 8, 1941 (*16723*); Elwha River, Olympics, Wash., Sept. 27, 1941 (*17336*); Olympic Hot Springs, Oct. 2 (*17538*); Mt. Angeles, Oct. 4 (*17549*), and Storm King Mt., Olympic National Park, Oct. 16, 1941 (*17963*).

Observations: The identity of this species puzzled me for a long time, but since Singer (1942) corrected Murrill's erroneous statements in regard to the spore size and shape, it becomes apparent that my collections belong here. The species is so abundant in the Puget Sound region that a collector would have difficulty overlooking it. It has a slight superficial resemblance to *Clitocybe atrialba* to which Murrill compared it, but cannot be considered closely related. In its spore characters it resembles *C. inornata* somewhat but after studying abundant fresh material of both species, I have been unable to note any additional resemblance. Macroscopically *C. avellanealba* reminds one of *C. clavipes,* in fact, the two are quite similar in all except spore characters.

The avellaneous color emphasized by the name was not apparent in my specimens until they were quite well faded and then it was not really char-

acteristic. Dark fuligineous, the other color term used by Murrill, is much more accurate.

University Herbarium, University of Michigan
Ann Arbor, Michigan

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JUNIPERUS VIRGINIANA, J. HORIZONTALIS AND J. SCOPULORUM—I. THE SPECIFIC CHARACTERS[1]

NORMAN C. FASSETT

This study is based primarily on the writer's collections and photographs, made in the region from Maine to Montana, and south to Georgia, Arkansas and Colorado, and financed in large part by the Wisconsin Alumni Research Foundation. Much of the material in the Arnold Arboretum, the Gray Herbarium, the New York Botanical Garden, Iowa State College, and the University of Wisconsin has been examined, and friends in Kansas, Oklahoma, Missouri, Texas, and New Mexico have contributed mass collections, most of which will be discussed. Mass collections have been made, consisting of a small twig from each tree in a colony, and representing many more individual trees than are present in any herbaria; these collections have been of particular value in solving problems of variation within a colony as compared to the variation within a species. Data have been taken from each individual and tabulated for the colony; tables 1, 2, 4, and 6 are representative of these tables.

Because of the remarkable variation in most colonies of these species, because a colony may consist of one variable species or of two species growing together, with or without intermediates, and because photographs of individual trees are often essential for an understanding of a colony, the writer feels that he is not competent to hold an opinion concerning species in regions where he has not collected, photographed and studied trees in the field. Therefore, *J. silicicola* (Small) Bailey, from the southern states, and *J. lucayana* Britton, from the West Indies, are not considered here, and the southern limits of *J. virginiana* are left indefinite. Likewise the European *J. Sabina,* which in some of its phases is indistinguishable, in the herbarium, from the American *J. scopulorum,* is omitted from consideration because the writer has not studied it in the field. In fact, *J. Sabina,* as represented in the Arnold Arboretum, may have leaves overlapping or not overlapping, with tips obtuse or acuminate, and even with erose margins, while the peduncles may be mostly straight or up to 88 per cent curved; these are characters which separate species in North America.

Juniperus virginiana, J. horizontalis and *J. scopulorum* are distinguished from the other species of North America north of the Gulf States by having opposite, scale-like, entire-margined leaves, and blue fruits.

[1] Publication of the tables was assisted by the Lucien M. Underwood Memorial Fund.

TABLE 1. *Ten individuals of J. virginiana from Quincy, Michigan.*

Width in μ of epidermal cells of leaves							Peduncles			Length in mm. of cones							
5	10	15	20	25	30	Av.	Hooked	Straight	% hooked	4	4.5	5	5.5	6	6.5	7	Av.
8	18	3	1[a]	.....	.....	9.5	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
8	16	5	1	.....	.....	9.8	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
5	17	8	.....	.....	.....	10.5	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
6	14	10	.....	.....	.....	10.7	0	14	0	3	6	2	.....	.....	.....	.....	4.5
2	20	8	.....	.....	.....	11.0	4	7	36	3	5	2	.....	.....	.....	.....	4.5
4	17	7	2	.....	.....	11.2	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
2	16	12	.....	.....	.....	11.8	0	6	0	.....	1	2	3	.....	.....	.....	5.1
3	13	14	.....	.....	.....	11.8	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
2	16	10	2	.....	.....	12.0	0	6	0	.....	1	5	.....	.....	.....	.....	4.8
1	17	11	1	.....	.....	12.0	1	13	7	.....	.....	.....	.....	.....	.....	.....	.....
41	164	88	7	.....	.....	11.0	5	46	10	6	13	11	3	.....	.....	.....	4.7

[a] This line signifies: 8 cells were 5 μ wide, 18 cells were 10 μ wide, 3 cells were 15 μ wide, one cell was 20 μ wide, and the average for that individual tree was 9.5 μ.

There are three facts which have sometimes made the differentiation of these three species difficult.

First, the variation within a species is often more conspicuous than differences between species. Well-known, of course, is the striking difference between the juvenile, acicular type of foliage and the adult, scale-like type. While usually the acicular foliage is produced by the younger trees and the scale-like foliage develops later, individual trees show divergence from

TABLE 2. *Ten individuals of J. virginiana var. crebra from Hopkinton, Massachusetts.*

Width in μ of epidermal cells of leaves							Peduncles			Length in mm. of cones							
5	10	15	20	25	30	Av.	Hooked	Straight	% hooked	3	3.5	4	4.5	5	5.5	6	Av.
4	6	.....	.....	.....	.....	8.0	0	6	0	.....	.....	1	2	2	.....	.....	4.6
3	7	.....	.....	.....	.....	8.5	2	23	8	.....	.....	1	3	7	5	4	5.2
5	3	2	.....	.....	.....	8.5	0	35	0	.....	.....	.....	10	10	.....	.....	4.8
3	4	3	.....	.....	.....	10.0	1	18	5	1	.....	5	2	2	.....	.....	4.2
4	2	4	.....	.....	.....	10.0	1	9	10	.....	.....	.....	1	4	2	1	5.1
.....	9	1	.....	.....	.....	10.5	1	26	4	.....	1	7	9	3	.....	.....	4.4
2	5	3	.....	.....	.....	10.5	3	9	25	.....	.....	3	6	1	.....	.....	4.4
1	6	3	.....	.....	.....	11.0	.....	.....	.....	.....	2	1	2	2	3	1	4.7
1	5	3	1	.....	.....	12.0	1	9	10	.....	.....	.....	1	4	1	.....	5.0
.....	5	4	1	.....	.....	13.0	7	21	25	.....	2	16	10	4	.....	.....	4.2
23	52	23	2	.....	.....	10.2	16	156	9	1	5	34	46	39	11	6	4.6

TABLE 3. *Summary of 15 mass collections from Maine, New Hampshire, Vermont, Massachusetts, Connecticut, Virginia, Georgia, Michigan, Wisconsin, Mississippi, and Arkansas.*

Width in μ of epidermal cells of leaves						Peduncles			Length in mm. of cones								
5	10	15	20	25	Av.	Hooked	Straight	% hooked	3	3.5	4	4.5	5	5.5	6	6.5	Av.
277	1111	511	66	1	10.9	36	574	6	7	34	92	139	131	42	13	1	4.6

this system. *J. virginiana* f. *Bermerae* Macbride has only acicular foliage; the type belongs, incidentally, to var. *crebra* Fernald & Griscom, but a similar phenomenon occurs in typical *J. virginiana*, as well as in *J. horizontalis* and in *J. scopulorum*. On some trees twigs with acicular foliage are intermixed with those bearing scale-like foliage, and on others all the foliage is intermediate in form between the two types. Injury, especially by the rust *Gymnosporangium*, may cause local reversion to the acicular foliage.

Other differences may pervade a colony; for example, the proportion of width to breadth of the needles may vary greatly from tree to tree (Am. Jour. Bot. **30**: 472. 1943). *The variation within any colony is often as great as within the species as a whole;* this fact is fundamental to an understanding of the taxonomy of the group. The color of the foliage often shows as much variation within a colony as it does within the species, and the writer has Kodachrome pictures of *J. horizontalis* with a bright green individual

TABLE 4. *Ten individuals of J. horizontalis from Baileys Harbor, Wisconsin.*

Width in μ of epidermal cells of leaves						Peduncles			Length in mm. of cones						
10	15	20	25	30	Av.	Hooked	Straight	% hooked	4.5	5	5.5	6	6.5	7	Av.
5	19	4	2	.....	15.5	7	0	100	.....	.....	.....	.....	.....	.....	.....
8	12	9	1	.....	15.5	5	0	100	.....	.....	.....	.....	.....	.....	.....
2	17	9	2	.....	16.8	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
8	10	6	5	1	16.8	3	1	75	.....	1	1	2	.....	.....	5.6
4	11	14	1	.....	17.0	7	0	100	.....	.....	.....	.....	.....	.....	.....
2	16	9	3	.....	17.2	15	0	100	.....	.....	3	4	1	.....	5.9
2	14	9	5	.....	17.8	6	1	86	.....	.....	.....	3	1	2	6.4
3	6	16	5	.....	18.8	4	3	57	.....	1	1	3	.....	.....	5.7
1	9	13	7	.....	19.3	6	3	67	.....	1	1	.....	1	1	5.8
1	3	19	6	1	20.5	3	0	100	.....	.....	.....	.....	.....	.....	.....
36	117	108	37	2	17.5	56	8	87	.....	3	6	12	3	3	5.9

TABLE 5. *Ten herbarium sheets of J. horizontalis from the Province of Quebec, in the herbarium of the Arnold Arboretum.*

Width in μ of epidermal cells of leaves					Peduncles			Length in mm. of cones							
10	15	20	25	Av.	Hooked	Straight	% hooked	4	4.5	5	5.5	6	6.5	7	Av.
13	11	6	.....	13.8	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
11	15	3	1	14.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
11	13	6	.....	14.2	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
5	22	3	.....	14.7	28	10	74	.....	5	13	4	4	.....	2	5.3
5	20	5	.....	15.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
4	18	7	1	15.8	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
1	23	6	.....	15.8	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
5	16	6	3	16.1	21	4	84	1	.....	13	6	2	.....	.....	5.3
2	16	11	1	16.8	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
3	14	8	5	17.5	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
60	168	61	11	15.4	49	14	78	1	5	26	10	6	.....	2	5.3

growing next to a glaucous one; they look more different than do many distinct species of *Juniperus*, but glaucous individuals seem to grow throughout the range of the species, in company with the ordinary bright green ones. Similarly striking are the shades of green of *J. virginiana*, varying within a single patch to colors bordering on the browns and purples. Shape of tree, size of fruits, etc., may vary throughout a colony, or in other cases there may be local phases. But most of these forms must be considered only as individual variations.

TABLE 6. *Ten individuals of J. scopulorum from Mammoth Hot Springs, Yellowstone National Park, Wyoming.*

Width in μ of epidermal cells of leaves						Peduncles			Length in mm. of cones							
5	10	15	20	25	Av.	Hooked	Straight	% hooked	4	4.5	5	5.5	6	6.5	7	Av.
.....	14	12	2	2	13.7	18	21	46	1	.....	1	5	.....	.....	.....	5.2
1	7	12	8	2	15.2	1	6	14	.....	.....	.....	1	4	1	1	6.1
1	6	14	9	.....	15.2	9	7	56	.....	.....	.....	1	2	3	.....	6.2
.....	3	19	8	.....	15.8	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
.....	2	20	8	.....	16.0	23	15	61	.....	.....	1	3	3	1	1	5.9
.....	6	14	8	2	16.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
.....	5	16	7	2	16.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
1	2	14	13	.....	16.5	15	17	47	.....	.....	2	3	7	.....	.....	5.7
.....	3	14	12	1	16.8	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
.....	2	15	11	2	17.2	8	17	32	3	4	6	.....	1	.....	.....	6.0
3	50	150	88	11	15.8	74	83	47	4	4	11	13	17	5	2	5.6

Second, the specific characters are often fully as variable as any others, but they vary in a different way and show reliable statistical differences. For example, straight peduncles may be found on *J. horizontalis* and hooked ones on *J. virginiana*, but these species may be distinguished by the fact that *J. virginiana* has a third or fewer of its peduncles hooked, while *J. horizontalis* has two-thirds or more of them hooked. Observation of a single peduncle is meaningless; examination of 10 peduncles will usually serve for positive identification. Diagnostic characters in width of epidermal cells of leaves, size of cones, seeds per cone, and in the markings on the seeds, are all of this statistical nature.

Third, two species may occur together in a colony, and hybrid swarms may or may not be formed. Hybrid swarms of *J. virginiana* and *J. horizontalis* occur on the coast of Maine and in the Driftless Area. In central and western South Dakota, and in northwestern Nebraska, the range of *J. virginiana* overlaps with that of *J. scopulorum*, and any colony may show a confusing set of interspecific variations. In the Big Horn Mountains, and about Banff, and probably elsewhere, *J. scopulorum* and *J. horizontalis* both grow, and they present some puzzling recombinations of specific characters, perhaps as a result of hybridization. These areas will, it is hoped, be discussed in subsequent papers.

The following specific characters are valid *except* in those regions, just listed, where two species grow together. Most of the statements below must be understood as having the limitation: *except in regions where two species occur.*

Character 1. The main trunk of *J. virginiana* is always erect, that of *J. horizontalis* is creeping, and that of *J. scopulorum* is erect or divided into several more or less ascending trunks. The rounded crown of *J. scopulorum*, originally used as a specific character,[2] is not always characteristic. When individual branches of *J. horizontalis* are infected with *Gymnosporangium* they may be erect and bear needles of the acicular type.

Character 2. The epidermal cells of the leaves of *J. virginiana* are from 5–20 μ wide, mostly 10 μ, those of an individual plant averaging 9–12 μ; in *J. horizontalis* they are 10–30 (rarely 5 or 35) μ wide, mostly 15 μ, averaging 13–19 μ; in *J. scopulorum* they are 5–25 μ wide, mostly 15 μ, averaging 15–19 μ. These measurements are perhaps the best means of distinguishing *J. virginiana*, in the herbarium, from the other two species. Yet the width of epidermal cells is very variable on each leaf; it is the mode and the mean which are diagnostic. For this reason it is necessary to measure a number of cells on each plant (thirty appears to be sufficient) and find the average. In this study, examination was made with a compound microscope, using a 32-mm. objective giving an enlargement of about 70 times; by means of a

[2] **Sargent**, Gard. & For. 10: 420. 1897.

camera lucida the width of each cell was marked on the edge of a sheet of paper, then measured with a scale previously prepared from a micrometer slide with the same microscope and camera lucida. Cells were measured to the nearest 5 μ, grouped in classes of 5, 10, 15, 20, 25, or 30 μ each, and averaged. Results for characteristic colonies are shown in tables 1, 2, 4, and 6, those for a large number of individuals totalling nearly 2000 individual cells in table 3, and for the herbarium material from a single region in table 5. It will be seen that all averages for individual plants show agreement, probably within the variation which might be expected from the relatively small numbers of cells measured per individual; indeed, when three individuals from one clone of *J. horizontalis* were measured they showed about as much variation as do the different plants of a colony. Here the variation within the species is not only equalled by the variation within the colony, but is nearly equalled by the variation within each individual.

Character 3. In *J. virginiana* each leaf nearly always overlaps the leaf directly beyond it; in *J. horizontalis* the leaves usually overlap; in *J. scopulorum* they rarely overlap, so that the exposed portion of each leaf usually appears 4-sided. The amount of overlapping of leaves may vary on any twig, particularly in *J. horizontalis*, where there is sometimes lack of overlap after the fashion of *J. scopulorum*. The principle, stated in the fourth paragraph of this paper, that variation within the colony may equal the variation within the species, may be observed by study of the amount of overlap of leaves on many twigs from one colony, as illustrated in Am. Jour. Bot. **30**: 472. 1943; in figure 17 of that paper, for example, some leaves barely overlap while others overlap a considerable distance.

Character 4. The apiculate leaf-tips of *J. horizontalis* are variable in length, but always present. They are occasionally present on the acute to acuminate leaf-tips of *J. virginiana;* a large colony of that species at Janesville, Wisconsin, is exceptional in that the leaves are apiculate on all of the 37 individuals with scale type foliage which were sampled. The leaves of *J. scopulorum* are subacute to very blunt, very exceptionally with tips which might be called subapiculate. The acicular leaves of all three species are pointed in about the same manner.

Character 5. While the foliar glands of *J. virginiana* and *J. horizontalis* rarely attain the size of those of *J. scopulorum*, occasional large individuals preclude the use of length as a diagnostic character. But the length of the gland of *J. scopulorum* equals or usually exceeds the distance from the gland to the tip of the adult type of leaf, while in the other two species the gland is always much shorter than that distance.

Character 6. Some of the manuals describe *J. virginiana* as having cones on straight peduncles, and *J. horizontalis* as having hooked or recurved peduncles. As a matter of fact, both straight peduncles and recurved

peduncles may be found on all three species, but the proportions of each are diagnostic. In *J. virginiana* the peduncles are all straight or up to 33 per cent curved; in *J. horizontalis* they are 60–100 per cent curved; in *J. scopulorum* they are 8–70 per cent curved. This character, it may be observed, will always serve to differentiate fruiting specimens of *J. virginiana* and *J. horizontalis*, and will sometimes serve to distinguish *J. scopulorum* from one of the other two species.

Character 7. *J. scopulorum* generally takes two years for fruit to mature, *J. virginiana* but one year; this was originally made the principal distinction between these two species.[3] As has been recently pointed out,[4] this does not always hold true, and if it did, it would be at best a weak specific character. Many specimens of *J. horizontalis* show two generations of fruit, but the writer has been unable to determine whether or not this is always the case.

Character 8. The fruits of *J. virginiana* average smaller (2.0–6.5, mostly 3.5–5.5 mm. long) than those of the other two species (4.5–8.0, mostly 5.5–6.5 mm. in *J. horizontalis* and 4.0–9.0, mostly 5.5–6.0 mm. in *J. scopulorum*), but individual fruits of *J. virginiana* may be larger than some individuals of the other two species. That this is a statistical difference, requiring the measurement of many fruits to identify a specimen, is shown in tables 1–6.

Character 9. The number of seeds in a cone was shown by Blake[5] to be different in *J. virginiana* and *J. horizontalis*. Again, the character is statistical; the usually limited supply of cones on a specimen, and the time consumed by their dissection, make this character less practical than most of the others here listed. The present writer's observation is that *J. virginiana* has 1–3 seeds per cone, *J. horizontalis* (1–) 2–6 seeds per cone, and *J. scopulorum* 1–4 seeds per cone.

Character 10. The long, deep pits on most (but not all) of the seeds of *J. scopulorum* serve to distinguish this from the other two species. Nearly all the seeds of *J. virginiana* are pitted;[6] about half the seeds of *J. horizontalis* are pitted. Seeds are best prepared for examination by picking the cones to pieces then dipping the seeds in alcohol and rolling between the fingers to remove adherent resin.

The facts outlined above have been derived from the study of mass collections from the following localities:

J. virginiana: MAINE; York Corner. NEW HAMPSHIRE; Hampstead, Derry. VERMONT; Colchester. MASSACHUSETTS; Newbury, Bedford, Hopkinton. CONNECTICUT; Portland. NEW JERSEY; Pompton Lakes, Darlington. VIRGINIA; Strasburg. WISCONSIN; Dykesville, Hartland, Janesville, Coon Valley. MICHIGAN; Quincy. TENNESSEE; Bradford. GEORGIA;

[3] **Sargent,** Gard. & For. **10**: 420. 1897.
[4] **Morton,** Rhodora **43**: 347. 1941.
[5] **Blake,** Rhodora **12**: 218. 1910.
[6] See Am. Jour. Bot. **30**: 474. 1943.

Cartersville. MISSISSIPPI; Corinth. MISSOURI; Gray Summit (collected by Dr. Edgar Anderson). FLORIDA; Santa Rosa Island. KANSAS; Sedan (collected by H. A. Stephens & L. H. Shinners), Riley Co. (collected by Frank C. Gates). ARKANSAS; Busch.

J. horizontalis: MAINE; Monhegan Island, Tenents Harbor. ONTARIO; Great Cloche Island, Little Current. MICHIGAN; Point Seul Choix. WISCONSIN; Baileys Harbor. WYOMING; Ranchester.

J. scopulorum: SOUTH DAKOTA; Upper Tunnel in the Bad Lands, Wasta, Interior. MONTANA; Bridger. WYOMING; Mammoth Hot Springs in Yellowstone Park, Burris.

In addition, the sheets of *J. horizontalis* and of *J. scopulorum* in the Arnold Arboretum, of *J. horizontalis* from Newfoundland in the Gray Herbarium, and of *J. virginiana* in the herbaria of the University of Wisconsin, Kansas State College, the University of Minnesota, and North Dakota Agricultural College, were examined for the characters listed above.

Certain representative data concerning three of these characters have been selected and presented in tables 1–6. In table 1, the epidermal cells in this colony of *J. virginiana* are seen to vary on each individual from 5 μ to 15 or 20 μ in width, and the averages to vary from 9.5 to 12.0 μ per individual. Since each average represents but 30 cells its significance is limited. The average of the 300 cells measured for the colony is 11.0 μ. In table 2, there is even a greater spread in averages for each plant, but the average for the colony is 10.2 μ, reasonably close to that in table 1. The lowest average for a colony of *J. virginiana* was 10.0 μ, and the highest 11.4 μ. For table 3, the measurements of nearly 2000 cells were averaged, to give a figure of 10.9 μ. The mode for every colony, and for every individual, is 10 μ in *J. virginiana*. Turning to *J. horizontalis*, where the mode for the individual is usually 15 and rarely 20 μ, we find in table 4 a range of 15.5–20.2 μ for averages of individual plants, with an average of 17.5 μ for the 300 cells measured from the colony; in table 5, representing 10 herbarium sheets from a single province of Canada, the individual plants range from 13.8–17.5 μ average widths, and the grand average is 15.4 μ. So, the results are the same whether we take 10 individuals from one patch, or 10 individuals from different localities; this illustrates the principle stated in italics in the fourth paragraph of this paper. The six mass collections of *J. scopulorum* showed similar variability of individuals and uniformity of colonies, therefore but one is described (table 6).

Examining now the character of straight vs. hooked cone-bearing peduncles, we find again considerable variation between individuals and uniformity of colonies. In table 2, the count, for the third plant in the list, of 35 straight peduncles against no curved ones is fair indication of nearly or quite complete lack of curved peduncles, yet on other twigs from the same colony may be found as many as one fourth of the twigs hooked. But, as was the case when epidermal cells were studied, the averages for the two colonies described in tables 1 and 2 are very similar (10 and 9 per cent, respectively), and close to what is found when 600 peduncles are examined (table 3). And again, variable as are the percentages for individuals of

J. virginiana, they never approach those of the equally variable *J. horizontalis* (tables 4, 5). The averages for individual trees of *J. scopulorum* overlap the higher figures of *J. virginiana* and the lower ones of *J. horizontalis*, but *J. scopulorum* is amply distinct from the others on a different set of characters.

SUMMARY

A study based on mass collections made throughout much of the ranges of *Juniperus virginiana*, *J. horizontalis*, and *J. scopulorum*, as well as on herbarium material, shows that these three species are always clearly recognizable on a number of characters, except when two species grow together, that the variation within each species is often more conspicuous than, but never as constant as, the variation which separates species, and that many of the distinguishing features are, while perfectly practical for the taxonomist, statistical in nature.

DEPARTMENT OF BOTANY, UNIVERSITY OF WISCONSIN
MADISON, WISCONSIN

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TAXONOMY AND DISTRIBUTION OF THE GENUS CERCIS IN CHINA[1]

HUI-LIN LI

The genus *Cercis* is widely distributed in the North Temperate Zone and, because of its discontinuous distribution, occurring as it does in southeastern Europe and adjacent parts of Asia, in eastern Asia, and in North America from New England to Michigan and California southward to Mexico, it is of special interest to the phytogeographer. This is a rather remarkable case of discontinuous distribution. It has been accepted that there is a lesser development of the genus in eastern Asia than in the other Eurasian and North American centers, with but two or three species occurring within a restricted area in central China. However, recent botanical explorations in China have increased the number of species as well as the geographic range of the genus in China, which involves a certain revision of our previous concepts of the group. The genus is a small one, a total of about twenty-five species having been described, more than one-third of which have been reduced to synonymy. It is suspected that too many species have been described from North America, for of the seven species proposed by Greene in 1912, Small in 1933 recognized none from the southeastern United States (at least two being from the range of Small's *Flora of the Southeastern United States*), while Jepson in 1936, in his *Flora of California*, reduced both of Greene's Californian species to *Cercis occidentalis* Torr. There are probably not more than about a dozen valid species of the genus now known.

This taxonomic study of the Chinese species is based on the material preserved in the herbarium of the Arnold Arboretum of Harvard University. Five species are recognized: *Cercis racemosa* Oliver, *C. Chuniana* Metcalf, *C. chinensis* Bunge, *C. Chingii* Chun, and *C. pauciflora* Li; and these are enumerated below with citations of all specimens examined. Among these, four are of rather limited occurrence. Only one, *C. chinensis* Bunge, is widely distributed in China, and in this respect can be considered as the counterpart of *C. Siliquastrum* L. of southern Europe and *C. canadensis* L. of North America.

Cercis chinensis Bunge occurs throughout the temperate regions of China from southern Kansu and southern Shensi in the northwest, eastern Sikang and northwestern Yunnan in the southwest, to northern Kwangsi in the

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south, Fukien, Chekiang, and Kiangsu along the coast in the east, and finally to Shantung and Honan in the northeast. Within this great circle there are only two provinces, Kiangsi and Hunan, wherein the species is not definitely known to occur.

It is evident, from the number of collections made and the data in the form of field notes, that this species occurs most abundantly in the hilly districts of western Hupeh and eastern Szechuan. Wilson, ex Craib in

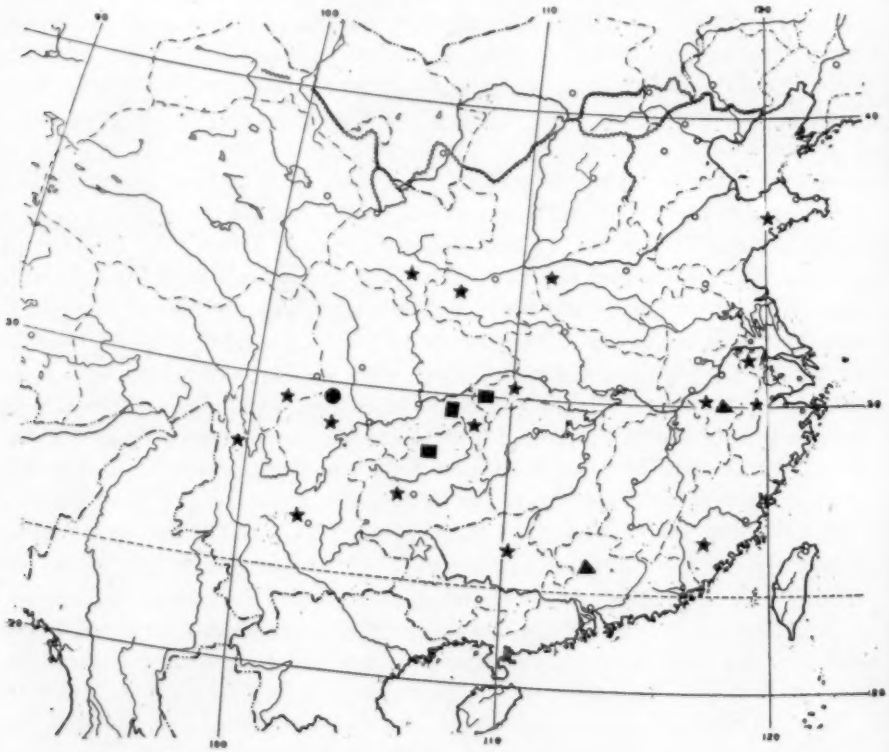

FIG. 1. Distribution of *Cercis* in China: Squares, *C. racemosa;* outlined star, *C. Chuniana;* solid stars, *C. chinensis;* triangles, *C. Chingii;* circles, *C. pauciflora.*

Sargent, Pl. Wils. **2**: 87 (1914), notes that it is very common in the latter regions. This area may thus be considered as the center of development of the species. The northern extension of the species is along the Tsinling Range, and thus 35° N is the approximate northern limit of its range. However, it is cultivated farther north in Peiping, which is about 40° N, and it is from the plants cultivated in that city that Bunge first described the species. It is also cultivated in various other localities in China from Peiping southward to Canton, and also in Japan, but is not indigenous to the latter

country. The natural habitat of the species is limited in eastern China to the hills along the western borders of the coastal provinces Kiangsu and Chekiang. In the south it is found in Fukien, northern Kwangsi, Kweichow, and northern Yunnan, with 24° N as its approximate southern limits. It is not found, except in cultivation, in the tropical southern part of Kwangtung, Kwangsi, and Yunnan; it is not recorded from Hainan, nor from Indo-China. In the west it occurs along the eastern border of the great Tibetan Plateau, in the mountains of northwestern Yunnan and eastern Sikang. Apparently the Himalayas and the Tibetan Plateau stand in between the species of eastern and central Asia, for continuous distribution of the genus in Asia does not exist.

In eastern Asia, this species is essentially a temperate-zone tree occurring in hilly districts. It is found in open country, and in thickets and woodlands, generally along the slopes up to an altitude of 1200 m., although in the high plateau in farther western China, it may occur at slightly higher elevations.

The other four species are all restricted in distribution. *Cercis racemosa* Oliver is common in western Hupeh especially in Fang Hsien, where, according to Wilson, it occurs in moist woods between an altitude of 1200–1800 m., at a higher level than *C. chinensis* Bunge. The only other collections of this species noted are from eastern Szechuan, adjacent to western Hupeh, and in northwestern Kweichow, also close to Szechuan. *C. Chuniana* Metcalf is localized in the northern part of Kwangsi Province. This is a tree of about 10 m. high, found in open woods at an altitude of about 1200 m. *C. Chingii* Chun has been recorded from two different localities, one in southern Anhwei, and another in northern Kwangtung. *C. pauciflora* Li is known only from Mount Omei in Szechuan Province, where it occurs at an altitude between 800 to 1600 m. (fig. 1).

KEY TO THE CHINESE SPECIES OF CERCIS

A. Inflorescences racemose, peduncles 2.5–10 cm. long.
  B. Leaves ovate-reniform to suborbicular, symmetrical; peduncles slightly puberulous ..... 1. *C. racemosa.*
  BB. Leaves rhomboidal-ovate, inequilateral; peduncles glabrous ..... 2. *C. Chuniana.*
AA. Inflorescences clustered, not pedunculate.
  B. Leaves ovate-reniform to suborbicular, large, about 7–15 cm. across; flowers preceding the leaves.
    C. Pods thin, to 15 cm. long and 1.5 cm. broad, winged along one margin, straight and usually not dehiscent ..... 3. *C. chinensis.*
    CC. Pods coriaceous, to 6.5 cm. long and 1.2 cm. broad, dehiscent, the valves twisted, not winged ..... 4. *C. Chingii.*
  BB. Leaves subtriangular-ovate, small, to 6 cm. long and 5 cm. broad; flowers appearing with the leaves ..... 5. *C. pauciflora.*

1. CERCIS RACEMOSA Oliver in Hook. Ic. **19**: *pl. 1894.* 1889; Diels, Bot. Jahrb. **29**: 409. 1900; Schneider, Ill. Handb. Laubh. **2**: 4. *f. 2 a, b, 3 a.* 1907; Craib in Sargent, Pl. Wils. **2**: 88. 1921; Chun, Chinese Econ. Trees 180.

1921; Rehd. Man. Cult. Trees Shrubs 482. 1927; ed. 2. 485. 1940; Stapf, Bot. Mag. **156**: *pl. 9316*. 1933.

HUPEH: Western Hupeh, *E. H. Wilson 607* (Veitch Expedition), May, 1900; Fang Hsien, *E. H. Wilson 314*, May, 1907 (flowers), Aug., 1907 (fruits), Sept., 1907 (fruits), a tree 15–30 ft. high, with much-branched head, in woods, alt. 4–5500 ft., flowers rosy pink; same locality, *E. H. Wilson 707*, Oct., 1907, a tree 10–35 ft. high, in woods, alt. 4–5500 ft. SZECHUAN: Wushan, *A. Henry 5602* (ISOTYPE). KWEICHOW: Pichieh, *Y. Tsiang 8989*, Sept., 1930, a large shrub 20 ft. high, along road side, in fruit.

This species is strongly characterized by the loosely racemose inflorescences (about 2.5–10 cm. long), and the leaves hairy on the under surfaces.

Wilson notes (ex Craib, l.c.) that "This remarkable tree is fairly common in the moist woods of central Fang Hsien between 1200–1800 m. alt., but is extremely rare elsewhere in western Hupeh and has not been reported from any other province. It is a low-growing tree, seldom, if ever, more than 12 m. tall, with a simple trunk from 1 to 2 m. in girth near the ground, and a widespreading head from 4 to 10 m. though of relatively thin branches. . . . It is essentially a cool temperate tree and in western Hupeh always at higher altitudes than *C. chinensis* Bunge."

The type locality, as noted above, is in the eastern part of Szechuan closely adjacent to western Hupeh. The Kweichow plant cited above is a fruiting specimen with the pods detached, but the hairy leaves clearly indicate that *C. racemosa* Oliver is represented. The species occurs in cultivation in some parts of the United States and in Europe.

2. CERCIS CHUNIANA Metcalf, Lingnan Sci. Jour. **19**: 551. *f. 2*. 1940.

KWANGSI: Northern Luchen, near the border of Kweichow, *R. C. Ching 6188* (TYPE), June, 1928, a tree 30 ft. high, in open woods, alt. 3800 ft., pods purplish, drooping; Shuenyuen, *Z. S. Chung 81566*, May, 1936, a tree 10 m. high, in woods, pods young, red; Chuen Yuen, *Z. S. Chung 81984*, June, 1937, along stream side, pods 9–10 cm. long, seeds 3–8.

Metcalf did not have flowers when he described the species, stating: "Inflorescences of small racemose branchlets with usually two–five fruits." In *Chung 81566*, there are inflorescences bearing very young fruits. These racemes measure 3.5–5 cm. long and bear many (10–15) flowers. The racemes usually become much shorter in the fruit, as is also true of those of *Cercis racemosa* Oliver.

In the racemose inflorescences, this species is allied to *Cercis racemosa* Oliver. But the peduncles, which Metcalf did not note, are glabrous in *C. Chuniana* Metcalf but puberulous in *C. racemosa* Oliver. Moreover, *C. Chuniana* Metcalf differs from Oliver's species in the pods being only very narrowly winged along one margin and the leaves being rhomboidal-ovate and very unequilateral. In the pronouncedly unequilateral leaves, a character that was not noted by Metcalf, this species is distinct and unique in the genus.

3. CERCIS CHINENSIS Bunge, Mém. Sav. Êtr. Acad. St. Pétersb. **2**: 95. 1833; Hemsley, Jour. Linn. Soc. **23**: 213. 1887; Pampanini, Nuov. Giorn. Bot. Ital. II. **17**: 393. 1910; Craib in Sargent, Pl. Wils. **2**: 87. 1914; Chun, Chinese Econ. Trees 178. *pl. 66*. 1921; Small, Addisonia **6**: 33. *pl. 209*. 1921; Chung, Mem. Sci. Soc. China **1**: 106. 1924; Rehd. Jour. Arnold Arb. **7**: 152. 1926; Rehd. & Wils. op. cit. **8**: 127. 1927; Chun, Sunyatsenia **1**: 253. 1934.

Cercis japonica Sieb. ex Planch. Fl. Serres **8**: 269. *pl. 849*. 1853.

Cercis glabra Pampanini, Nuov. Giorn. Bot. Ital. II. **17**: 393. *f. 9*. 1910; Bull. Soc. Tosc. Ortic. **36**: 215. *f. 16*. syn. nov. 1911.

HOPEI: *T. N. Liou 1228, 1229*. SHANGTUNG: *C. Y. Chiao 2430, 2818*. KIANGSU: *R. C. Ching 4884, Ching & Tso 417, Y. L. Keng 2597, C. L. Tso 945*. CHEKIANG: *Tang & Hsia 459, Y. L. Keng 847*. ANHWEI: *A. N. Steward 2306, R. C. Ching 3145*, HONAN: *J. Hers 291, 404, 525, 579*. SHENSI: *J. Giraldi s.n., J. Hers 2443, 3042, G. Fenzel 254*. KANSU: *F. N. Meyer 1642, 1935*. HUPEH: *A. Henry 7662, C. Sylvestri 1049, 1052* (SYNTYPE of *C. glabra* Pampanini, photo.), *3043, E. H. Wilson 501, 761, H. C. Chow 146, 593, W. Y. Chun 4143*. SZECHUAN: *A. Henry 7662 B, Bock & Rosthorn 1640, J. F. Rock 12041, W. P. Fang 10203, 22390*. SIKANG: *C. Y. Chiao 231*. YUNNAN: *G. Forrest 23223, 26273, 27194, J. F. Rock 8918, 9409, R. C. Ching 20583, K. M. Feng 2666, T. T. Yü 21075, W. C. Cheng 11000*. KWEICHOW: *H. Handel-Mazzetti 163, S. W. Teng 90029 A, 90029 B*. KWANGSI: *H. Fung 21190*. FUKIEN: *H. H. Chung 1243, 4833, 6972*.

Dunn and Tutcher, Kew Bull. Add. Ser. **10**: 90. 1912, credit this species to Kwangtung Province, Lienchow River, but this apparently represents a misidentification of *Cercis Chingii* Chun.

This species was first described by Bunge from cultivated plants in Peiping. It appears to be indigenous and widely distributed in temperate China. It is most abundant in western Hupeh and eastern Szechuan, which region may be considered as the center of distribution of the genus in general and of this species in particular. Wilson (ex Craib, l.c.) notes that "In western Hupeh and eastern Szechuan this beautiful flowering tree is very common but we never met with it wild west of the Red Basin of Szechuan. It occurs in open country, and in thickets and the margins of woods up to 1200 m. altitude. . . . This tree grows from 6 to 15 m. tall and has a moderately thick trunk clean of branches for half its height and a flattened rounded head." However, it has since been collected, as noted by the cited specimens above, in western Szechuan.

Wilson also notes "The flowers vary from pale pink to red pink and are produced in great numbers on all parts of the tree including the old branches and main trunk." In one of the collections cited above, *J. F. Rock 12041* from Ching-chuan, western Szechuan, it is indicated on the field notes: "flowers white." This is the only collection noted for white flowers.

It is observed that among the assorted specimens of the species from different localities, while in general being very uniform in their characters, slight variations are found in the length of the pods and the pubescence of the leaves and pods. In most instances the leaves are completely glabrous, while in others they are slightly pubescent along the veins near the base on

the lower surface. Pampanini (l.c.) referred some of Sylvestri's specimens from western Hupeh with their leaves slightly pubescent below to *C. chinensis* Bunge, and three other specimens with completely glabrous leaves as representing a new species, *C. glabra* Pamp. Wilson (l.c.) notes that he is doubtful if *C. glabra* Pampanini is anything more than a mere form of *C. chinensis* Bunge. Type material of Pampanini's species, including a photograph of *Sylvestri 1052*, fragments of leaves and fruits of *1052 a*, and fragments of flowers of *1051*, all from the Biondi Herbarium, Florence, are now available for study. It is found that such a variation in pubescence is common in other specimens and that age is also a factor. I do not consider that Pampanini's species is a valid one, and it is accordingly reduced to synonymy.

A more definite variation, in the pubescence of the pods, is, however, present. Among the fruiting specimens from the provinces of Chekiang, Anhwei, and Kiangsu, and the cultivated plants from Tsingtao, Shantung Province, and Peiping, Hopei Province, it is found that they are very finely pubescent throughout. But the ovaries of the few flowering specimens from these same regions appear to be glabrous as in the other specimens cited. The pods are also of relatively shorter length in general, but some have long pods; and short pods are not uncommon in plants from other regions. In other characters these plants agree exactly with plants from other regions except one specimen, *T. Tang & W. Y. Hsia 459*, that has leaves puberulous throughout on both surfaces and rather conspicuously so on the lower surface. It appears that a form geographically located in eastern China and cultivated elsewhere is represented. As Bunge described the species from plants cultivated in Peiping, it may be that this pubescent form is the typical form of the species while plants from other parts of China represent another form. However, it is felt that any attempt to divide this species into forms or varieties should be based on actual observations of living specimens in the field.

This species, like *Cercis racemosa* Oliver, is cultivated in Europe and in various parts of the United States, being hardy as far north as the southern part of Massachusetts.

4. CERCIS CHINGII Chun, Jour. Arnold Arb. **8**: 20. 1927; Rehd. & Wilson, Jour. Arnold Arb. **8**: 127. 1927; Chun in Hu & Chun, Ic. Pl. Sin. **2**: *pl. 82.* 1929, Sunyatsenia **1**: 253. 1934.

Cercis chinensis sensu Dunn & Tutcher, Kew Bull. Add. Ser. **10**: 90. 1912; non Bunge.

ANHWEI: East of Hweichow city, *R. C. Ching 3332* (TYPE), Sept., 1925, a fairly common gregarious shrub along roadsides in the open. Also recorded from northern Kwangtung (Chun, l.c., 1934).

In its general habit, this species closely simulates *Cercis chinensis* Bunge. It is characterized by the thickly coriaceous pod, which is not winged at the

margin, but is dehiscent, and its valves somewhat twisted, when mature. The seeds are few (3–6), about 7–8 mm. long and 4–5 mm. wide, and imbedded in a thick whitish spongy tissue.

Chun says that "According to the late Dr. Stapf, some other genus might be represented. The dehiscent pod of the species is anomalous in *Cercis*." However, it is noted that in some very mature fruits in *Cercis chinensis* Bunge, the pods also dehisce along one or both margins (examples: *Wilson 761* and *K. M. Feng 2666* cited under *Cercis chinensis* Bunge above).

5. **Cercis pauciflora** Li, sp. nov. Frutex, ramulis cinereis, ramulis novellis brunneis, elenticellatis; foliis chartaceis, longe petiolatis, subtriangulariter ovatis, 4–6 cm. longis, 5 cm. latis, acutis vel acuminatis, basi truncatis vel leviter subcordatis, supra viridibus, subtus pallidioribus, utrinque glabris, nervis primariis 5–7, gracilibus, supra subconspicuis, subtus elevatis, rete venularum utrinque subconspicuo; petiolo gracile, 2–2.7 cm. longo, apice leviter dilatato; floribus axillaribus cum foliis coetaneis, in ramis biennibus vel vetustioribus dispositis, 1–3-fasciculatis, bracteis ovatis minutis, 1.5 mm. longis, margine pubescentibus, pedicellis brevibus, saltem 3 mm. longis, glabris; calycis tubo cupuliformi, 4 mm. alto, 3 mm. lato, plus minusve irregulariter 5-lobato, lobis rotundatis, 1 mm. diametro, margine ciliatis; corolla rubra, vexilli ungui lineari 3 mm. longo, lamina reflexa oblongo-elliptica 4–5 mm. longa, maculis atrorubris adspersa, alis forma magnitudine similibus, patentibus, emaculatis, carina cymbiformi, apice rotundata, 1.1 cm. longa, ungui gracili brevi; staminibus 10, filamentis liberis, 8–9 mm. longis, antheris atrorubris; ovario plus minusve lineari, 3–4 mm. longo, glabro, stylo 6–7 mm. longo, stigmate inconspicuo.

SZECHUAN: Mount Omei, *Y. S. Liu 1700* (TYPE), Oct., 1937, a shrub, alt. 800–1600 m., in flower.

In its vegetative characters, this species is characterized by the relatively small, subtriangular-ovate leaves. It differs from *Cercis chinensis* Bunge, in addition to its vegetative characters, in the fewer (1–3) flowers in each cluster with much shorter pedicels (scarcely 3 mm. long). In *Cercis chinensis* Bunge the flowers are usually 4–10 or more together and the pedicels 1.5–3 cm. long. Moreover, the calyx-tube of *C. chinensis* Bunge is broadly open above with more or less triangular lobes which are generally not ciliate along the margins; while in this new species the calyx-tube is not broadened above, and its rounded lobes are distinctly ciliate along the margins. This new species is furthermore characterized by the fact that the flowers appear in the fall when the leaves are fully mature. This autumn flowering may be or may not be a regular condition, but nevertheless, the other characters of the plant indicate that an undescribed species is represented.

ARNOLD ARBORETUM, HARVARD UNIVERSITY
JAMAICA PLAIN, MASSACHUSETTS

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A PRELIMINARY CONSIDERATION OF THE BIOSYSTEMATY OF OXYCOCCUS

W. H. CAMP

The cranberries are a compact group of the Vaccinieae, with a circumboreal distribution. Any decision whether they should be treated as the separate genus *Oxycoccus*, or as a section, or subgenus of *Vaccinium*, must await a more thorough investigation of the phylogeny of the entire group than is now available. Certainly, if this group is to be recognized as a genus, then a number of genera should be segregated from within what is now the widespread and polymorphic genus *Vaccinium*. The fact that the name *Oxycoccus* has been used in the present title and discussion does not necessarily indicate that I have come to a personal decision on the matter; it was done primarily because the group is so treated in the majority of current European and American works.

For well over a decade I have been attempting to arrive at some satisfactory solution of the problems which the systematics of even this small group presents. During this time, several papers of considerable interest have appeared. One of these, by Porsild (1938), was a taxonomic treatment of the Canadian material, although it actually dealt with all recognized species. The other, by Hagerup (1940), contained a cytological analysis of the European populations, and something of their ecology. Recent work on certain American materials (Darrow, Camp, Fischer & Dermen, in preparation) brought to light additional information which warranted a further discussion of the group.

In his systematic treatment, Porsild recognized four species and one variety. An additional entity—"gigas"—was noted by Hagerup.[1] By combining these two treatments with the findings of the chromosome complements of American material as reported by Darrow, Camp, Fischer & Dermen, the following cyto-taxonomic picture of the group may be obtained:

Oxycoccus macrocarpus (Ait.) Pers.	2n = 24.
Oxycoccus microcarpus Turcz.	2n = 24.
Oxycoccus quadripetalus Gilib.—"typical" material	2n = 48.
Oxycoccus quadripetalus var. *microphyllus* (Lange) M. P. Porsild	2n = 48.
Oxycoccus ovalifolius A. E. Porsild[2]	2n = 48.
Oxycoccus "gigas"	2n = 72.

[1] Although Hagerup treated this entity as a binomial in the text, it was not set up in a manner entirely satisfactory to the present Rules of Nomenclature; where used in this discussion, the name will appear in quotation and without italics.

[2] Based on *Vaccinium oxycoccus* var. *ovalifolium* Michx., considered by Porsild—following a former decision of Robinson & Fernald (Rhodora 11: 54. 1909.)—to be an earlier

Having previously made field observations of the material in eastern North America, the mid-continent area, and in British Columbia, and thus—with the exception of *O. microcarpus*—having seen considerable amounts of the entities listed for North America growing under natural conditions, I was in general accord with Porsild's treatment when it appeared. However, the recent findings of the cytological condition within the cranberries—together with an increased understanding of the factors of speciation in the Vaccinieae in general—have led me to a preliminary consideration of the biosystematy of the group, the results of which are presented here. A definitive report must await the accumulation of considerably more data than are now available. In reviewing the situation, the following items should be noted, for they have played a prominent part in the interpretation of specific lines:

1. A considerable amount of controlled breeding work indicates that there are almost no sterility barriers between homoploid individuals and species in *Vaccinium* subgenus *Cyanococcus* (Darrow & Camp, in preparation). While it does not necessarily follow that the same situation is equally true in *Oxycoccus*, there are reasons to believe that such is the case.

2. A species which has arisen either through allopolyploidy or by hybridization is likely to be highly segregative. With time, various of the segregate forms may be differentially selected by environmental forces; that is, the segregate forms may be unequally successful in various habitats. A recent paper by Camp & Gilly (1943, p. 342–348) may be consulted for a more detailed discussion of the origin and development of alloploidic and mictonic species.

In reviewing the North American material, it is obvious that the diploids (*O. macrocarpus* and *O. microcarpus*) are easily separable, each reasonably homogeneous and, today, distributionally disjunct. On the other hand, when sufficient material is examined, the tetraploids are found to be variable, and to intergrade to the extent that the entities defined would seem to have doubtful significance; certainly, they are difficult to separate with any degree of precision. As a group, the American tetraploids not only occupy the region between the present diploids, but also overlap the ranges of both to a considerable extent. It is also considered to be significant that the bulk of the tetraploid population combines the characters of both diploids, whereas the extremes tend toward one diploid or the other, thus fulfilling the expected variability of a segregative population.

Since it seems likely that the individuals of the tetraploid population are interfertile and, as will be pointed out later, there is evidence that no more

name for *Vaccinium oxycoccus* var. *intermedium* Gray, a plant supposed to be of the American Pacific Northwest and northeastern Asia. It is doubtful whether a careful reanalysis of the types would indicate that they were precisely equal. Porsild's paper may be consulted for the general synonymy of the group.

than a generalized geographic segregation has taken place among them, it is thought best to consider them as members of a single species. The two diploid populations will be treated as distinct species. In order to expedite our later discussion, brief descriptions of the more easily visible diagnostic characters of these species are presented here together with a summary of their distributions, these given in detail only for North America.

OXYCOCCUS MACROCARPUS (Ait.) Pers. A diploid species (2n = 24).

Leaves flat or only slightly revolute, elliptic-oblong, apically rounded, 5–15 mm. long, 2–8 mm. wide. Flowers 2–6, solitary in the axils of the lower bracts or basal leaves of a normal leafy branch. Pedicels pubescent, bearing a pair of green bracts above the middle, or even subtending the fruit; bracts usually leaf-like, 2–4 mm. long (forms are known with minute, red, scale-like bracts 1.5 mm. long). Calyx lobes triangular-acute, sometimes obtuse. Fruit 10–20 mm. thick, generally deep pink to red, tartly acid.

Distribution: North America; estab. in Europe, probably first as a ballast waif, sometime prior to 1869. In North America: Minnesota, eastward to Newfoundland, south to Tennessee and North Carolina; reported by Small (Man. 1933) from Arkansas. Extensively cultivated in eastern North America and to some extent along the Pacific coast, where it is said to have escaped.

OXYCOCCUS MICROCARPUS Turcz. A diploid species (2n = 24).

Leaves strongly revolute, ovate when flattened, apically pointed, 2–6 mm. long, 1.5–2 mm. wide. Flowers 1–2(3), in a determinate inflorescence, the result of abortion of the vegetative portion of the branch. Pedicels glabrous, bearing a pair of red bracts below the middle; bracts scale-like, 0.5–1 mm. long. Calyx lobes relatively short, rounded to obtuse, or sometimes mucronate. Fruit 5–7 mm. thick, pink, insipid.

Distribution: Iceland, Europe, Asia, and northwestern North America; unknown from Labrador and Greenland. In North America: Alaska to British Columbia and Alberta (Queen Charlotte Isl., and in the mts. to about 51° Lat.), eastward to northern Saskatchewan and parts of the Northwest Territories. Reported by Porsild from the east shore of Hudson Bay.[3]

O. QUADRIPETALUS Gilib. A tetraploid species (2n = 48).

Leaves flat to strongly revolute, narrowly to broadly elliptic-ovate, apically acute to obtuse, or rarely somewhat rounded, 2–10 mm. long, 1.5–5 mm. wide. Flowers (1)2–3(4) in a determinate inflorescence, or at the base of a weak branch which soon dies, or at the base of a strong vegetative branch which continues growth. Pedicels pubescent (sometimes obscurely so), bearing a pair of red bracts below, at, or above the middle; bracts scale-like, 0.7–1.5 mm. long. Calyx lobes variable, generally obtuse or mucronate, sometimes rounded, or occasionally triangular-acute. Fruit 7–13 mm. thick, pink, or pink flecked with red dots or stripes, or sometimes red, sub-acid to acid.

Distribution: Europe, Asia, North America, and Greenland; unknown from Iceland. In North America: Alaska, eastward to Great Slave Lake and

[3] Although a logical extension of range, this material may yet prove to be a "microcarpoid" segregate of *O. quadripetalus*, the common form in Labrador and Greenland.

Labrador, south to n. California, n. Idaho, Minnesota, ne. Ohio, and New Jersey.

Since Porsild recognized two species (one with a variety) in what I now consider to be a single tetraploid species, it would be well to discuss this material in some detail. In the first place, one must assume from his discussion and key that the lack of a well developed pubescence on the pedicel and a relatively large leaf were the more important factors in the recognition of *O. ovalifolius*. In examining a fairly large series of plants I find some which answer the description of this species as delimited, but even in the Pacific Northwest much of the material has a conspicuous pedicellary pubescence and would seem better placed in the large-leafed phase of *O. quadripetalus*. Therefore, it would seem more expedient not to maintain *O. ovalifolius* as a species.

Let us consider, then, the entire tetraploid population and determine whether it can be broken down into subsidiary segments. If it can—and if the population is segregative—there should be a series of recognizable morphological groups. But first as to the method of analysis. Since the tetraploid population apparently has been derived in some manner from a combination of the diploids, we may legitimately use the contrasting characters of the diploids in making our separations. Among the more conspicuous of these, the following might be listed: "basal" vs. "determinate" inflorescence, "large" vs. "small" leaf,[4] pubescent vs. glabrous pedicels. It is naturally a matter of opinion whether these are the most important characters, but they are as obvious as any and will suffice to begin the study.

Although individual specimens may occasionally give trouble, the bulk of the tetraploid material can be easily separated into two groups, one with a "macrocarpoid" inflorescence, the other with a "microcarpoid" inflorescence.[5] Likewise, although somewhat more arbitrary, there are such marked differences in leaf size and shape that each of the groups already set up can be divided into "large-leafed" and "small-leafed" subgroups (again, "macrocarpoid" vs. "microcarpoid"). Since, by definition, the pedicels of this material are never completely glabrous, a further "macrocarpoid" vs. "microcarpoid" separation can be made only on the basis of dense pubescence vs. scattered or obscure pubescence. Again, by using arbitrary limits, additional separations may be made. Thus, if a sufficiently large amount of material is available, eight reasonably distinct classes of segregate forms may

[4] The terms "large" and "small" as used here in reference to the leaf do not mean size only; in this instance, differences in length-width ratios, and relative amount of surface involved in the revolute margin, as well as the actual over-all surface area, are the determining factors in the separation of the "large" ("macrocarpoid") and "small" ("microcarpoid") leaf types.

[5] A number of years ago, Fernald (1902) called attention to this type of variation.

be recognized, each yielding to identification by a key no more complicated or difficult than that used in the differentiation of many species.

Nor are the foregoing the only characters which might have been used. Among the items which are equally diagnostic of the basic diploids under detailed examination, and which certainly should be used in any future critical and definitive analysis, are the following: (1) length-width ratio of the corolla lobe; (2) type and distribution of pubescence on the anther filament; (3) the relative exsertion of the style at anthesis; (4) the shape of the calyx lobe; (5) the presence or absence of—and the relative conspicuousness of—the tuft of hairs at the apex of the calyx lobe; (6) the percentage of total surface involved in the revolute leaf margins; (7) the shape of the leaf apex; and even (8) the basic color and type of mottling on the fruit. With a brief search in any considerable amount of tetraploid material, individuals can be found which exhibit almost any combination of these characters. It is therefore obvious, when viewed in its entirety, that the tetraploid population has considerable morphological variability, indicative of a high degree of genetic segregation.

Our next problem is to determine whether sufficient selection pressure has been exerted on this segregative population to yield segments worthy of nomenclatural recognition. Frankly, I see no need to force the issue to its ultimate limits; the character of a segregative complex is such that we would soon find ourselves involved with the plants of a single locality, or even with individuals. Let us, therefore, for the present, pass over the lesser segregative groups and concentrate on the more generalized types, permitting a certain degree of complexity in each. We can, for example, consider the broadly defined "macrocarpoid" group of segregates—those with basal inflorescences, generally large and only slightly revolute leaves, and relatively pubescent pedicels—and, as a unit, consider its distribution. At first glance, it appears to be scattered, yet a closer examination reveals that it becomes a more prominent component of the tetraploid population as one goes southward.[6] Nor is it limited to the Pacific Northwest and northeastern Asia, with a few casual outliers in mid-continent North America, as was originally supposed. In some restricted areas in eastern North America it appears to be the dominant form; and in New Jersey, at the southern limit of its range, it makes up about half of the general population. Conversely, although the "microcarpoid" segregates are notably concentrated along the northern margin of the tetraploid population, excellent examples are known from as far south as Long Island, N. Y. Much the same situation prevails in the mid-continent area and in the Pacific region where occasional "microcarpoid" plants may be found near the southern border of the complex.

[6] An excellent example of the "macrocarpoid" phase of *O. quadripetalus* from California—labeled "*O. macrocarpos*"—may be found illustrated in a recent paper by Dayton (1938).

It is therefore apparent that the selective forces which brought about the geographical disjunction of the diploid populations of *O. macrocarpus* and *O. microcarpus* have also been operative within the tetraploid population. That the "macrocarpoid" and "microcarpoid" phases of the tetraploid have not achieved a greater degree of regional concentration and geographical isolation is probably to be explained by the fact that neither is genetically pure, each having a little of the other in its hereditary make-up. This may also explain why the tetraploid, *O. quadripetalus,* while occupying a much larger territory than either of its diploid ancestors, seems never to have been able to venture quite as far north as *O. microcarpus,* nor quite as far south as *O. macrocarpus.*

Let us now turn to the subspecific nomenclatural situation in *O. quadripetalus.* Because of its small leaves, there is a strong inclination to associate the "microcarpoid" phase with var. *microphyllus.* By arbitrary methods, the large-leafed phase (if limited to a "microcarpoid" type of inflorescence) might be taken to be the "typical" material. Using similar methods, we might conclude that var. *ovalifolium*[7] was the equivalent of the "macrocarpoid" phase. However, to follow such a nomenclatural scheme with any degree of precision would necessitate the re-examination of type specimens, a thing which is impossible under present conditions. Furthermore, even if a complete set of type specimens were available the problem would not be solved, for the complex nature of the tetraploid population—if we were to approach it in any critical manner—would preclude any but a most complicated nomenclature. Because of this fact, I would suggest, at least for the present, if the binomial *Oxycoccus quadripetalus* is not sufficient for some particular purpose, that there be appended to it some term as the "macrocarpoid phase," the "microcarpoid phase," or the "median phase," depending upon the more obvious of the morphological characters exhibited by the material. Thus any possible nomenclatural errors resulting from a hasty application of names would be circumvented, until such time as the type specimens are again available. In fact, even then, I cannot see where much is to be gained by attempting to make too fine subspecific nomenclatural distinctions in this particular population. To increase the subspecific names to what appears to be a critical minimum would require considerablly more material than is now available in herbaria, or is likely to accumulate in the near future and, even then, to do so might needlessly encumber the nomenclature of the species. On the other hand, to use the three most obvious subspecific groups, mentioned here as possibilities in *O. quadripetalus,* would simplify nomenclatural matters once the types were ascertained. But it must be admitted that such a procedure would result only in a broad systematic generalization lacking in critical details.

[7] The combination, as a variety, seems never to have been made in *Oxycoccus;* hence the "*um*" rather than the "*us*" ending.

Thus far, we have been dealing only with the North American material of *O. quadripetalus*. Although the specimens available to me from outside this region are not overly abundant they are sufficient to make certain observations. On examining all the available material of this species one thing was obvious: it could be separated with relative ease into the "macrocarpoid phase," the "median phase," and the "microcarpoid phase," not only in North America, but also in northeastern Asia, north-central Asia, and Europe.[8] It is therefore apparent that the population of *O. quadripetalus* is contant in one characteristic: *it is highly segregative throughout its range.*[9]

Having made some attempt to clarify the general picture of the two diploid species and the derived tetraploid, we may now turn to a discussion of the apparently sterile hexaploid, "gigas." The sterility of "gigas" appears to be caused by aberrancies during meiosis resulting in abortive pollen. So far, according to Hagerup, this material is known only from scattered stations in Denmark, Finland, and East Prussia. In looking back over my own field work in North America, I can recall occasional plants similar to *O. quadripetalus*, but somewhat coarser than usual, which seemed to be unfruitful. In going over the herbarium material, additional specimens have been found whose vegetative parts closely match those of the hexaploid plant illustrated by Hagerup. In various instances, these were either in flower ("gigas" flowers freely) or, if collected late in the season, bore no fruit. It is therefore probable that hexaploid plants are not unique in Europe, but also occur sporadically throughout the range of *O. quadripetalus* in Asia and North America.

Hagerup was somewhat of the opinion that these hexaploid plants were the result of hybridization between individuals of the tetraploid *O. quadripetalus* and the diploid *O. microcarpus*. Based on studies of the origin of hexaploids in *Vaccinium* (Camp 1942a; and Camp, in press), it would seem unnecessary to suppose that this is the case. In fact, it is unlikely. For the most part, the hexaploids of *Vaccinium* apparently have been derived out of tetraploid populations, sometimes highly heterozygous in nature. The mechanics seems to be the fusion of a normally reduced gamete with a non-

[8] Something of the morphological complexity of the European population may be gleaned by consulting a series of papers by Gleisberg (1919, 1922a, 1922b). A continuation of such analyses today would necessitate co-ordinate cytological studies, for Gleisberg considered *microcarpus* to be only a varietal manifestation of the tetraploid population, nor did he know of the presence of the hexaploid "gigas."

[9] On casual observation, one population would appear to be an exception; this is the material from Greenland, which seems to be mostly "microcarpoid." This, however, is no more than might be expected. Even so, after careful examination, it appears that there is considerably more variability in fundamental characters such as length-width ratios of the leaves, shape of the leaf apex, shape of the calyx lobes, and relative pubescence of pedicels than might have been anticipated, leading one to suspect that a larger series of specimens might uncover further segregate forms.

reduced gamete, thereby directly yielding a hexaploid individual. Nor would it seem necessary to speculate, as did Hagerup, on the problem of the dispersal of such material. There is ample evidence in *Vaccinium* that it is a repetitive process, and I see no reason to suppose that it happened only once in *Oxycoccus*, although it is admittedly interesting to consider the possibility of the European hexaploids as being parts of a single, widely dispersed clone. I fully expect that additional cytological work in Asia and North America will reveal other hexaploids. In fact, we should not be surprised if some of them prove to be fertile.[10]

There is perhaps one last point which might be brought up. That is the possible phyletic development within the group here under discussion. Of the known living diploids, *O. macrocarpus* seems to have retained the greatest number of primitive characters; in addition, it still persists in the region of the Southern Appalachian Mountains of North America, that great reservoir of Tertiary archetypes.[11] It is easy to suppose that *O. microcarpus* was derived directly from *O. macrocarpus* through reductions and tissue readjustments, the result of a series of mutations. The end product was a population considerably different from the ancestral form and able to migrate into new areas and persist under conditions which excluded the archetype.[12] This is an example of primary speciation.[13]

In considering the origin of the tetraploid population, there is no need to build up any complicated hypothesis as to its possible ancestors, for it exhibits no morphological characters which are not present in the two existing diploids. Therefore, we might conclude that it has arisen by a compounding of the heredities of *O. macrocarpus* and *O. microcarpus*. We have only to discuss the mechanics of its origin. Two alternative methods seem most likely:

1. Subsequent to the disjunction of the two diploid species, they were brought together along their margins by some climatic factor which forced a partial readjustment in range. Where they met, hybridization took place.

[10] Three American species of *Vaccinium* are known to be hexaploid and completely fertile (Darrow & Camp, in preparation; and Camp, in press); in fact, about half of the acreage of the present commercial plantings of blueberries in the United States is of hexaploid material.

[11] It is in this region where one also finds the relatively primitive *Vaccinium erythrocarpum* Michx. (= *Schollera erythrocarpa* Steud.; = *Oxycoccus erythrocarpus* Pers.; = *Oxycoccoides erythrocarpus* Nakai; = *Hugeria erythrocarpa* Small) an erect shrub with deciduous leaves, but with a type of flower quite similiar to that of *C. macrocarpus*. In the first paragraph of this paper it was mentioned that much yet needed to be done to establish satisfactory generic lines within the Vaccinieae.

[12] The advantages of a low-growing plant under subarctic conditions, where the ground may be swept almost free of snow, and where there is considerable browsing by large animals, has been mentioned in another place (Camp 1942b, p. 223, footnote 21).

[13] The differences between primary and secondary speciation were briefly discussed in a previous paper (Camp 1942a).

Then, out of this hybrid population there arose a series of fertile allotetraploids. After this, another shift in climate took place which again separated the diploids, leaving the allotetraploid population in possession of the area where it arose. This would be the nuclear population out of which the present *O. quadripetalus* has spread.

2. After the geographic disjunction of the diploids, autopolyploids arose in each, forming effective independent reproductive tetraploid populations. That derived from *O. microcarpus* would have been less effective in the far North than the diploid because of its larger size, and so might have persisted as an effective population by migrating southward. Thus the tetraploids of *O. microcarpus* could have come into contact with those of *O. macrocarpus* and hybridized with them, the result being the nuclear element of the present population of *O. quadripetalus*.

Prior to a more careful analysis of the situation, it is difficult to determine which of these methods was responsible for the origin of *O. quadripetalus*, for either would be likely to yield a population which was potentially segregative and superficially quite similar in appearance. A recent paper by Dermen and Bain (1941) indicates that polyploid material can be experimentally produced in *O. macrocarpus*. It might be supposed, if polyploids can be induced with a certain degree of ease, that they are likely to occur in reasonable abundance under natural conditions, not only in *O. macrocarpus*, but also in *O. microcarpus*. This has not yet been found to be the case for either.[14] However, the apparent absence of autotetraploids might be explained by assuming that they are "absorbed" by the tetraploid *O. quadripetalus* population about as fast as produced, since this species covers so much of the ranges of the diploids. This hypothesis might also be used to explain the concentration of the "microcarpoid" forms along the northern border of *O. quadripetalus*, and the similar concentration of "macrocarpoid" forms along its southern border. But, since *O. macrocarpus* is limited to eastern North America, this does not at all serve to explain the presence of the strong "macrocarpoid" element in the material of *O. quadripetalus* in western North America, Asia, and Europe; nor will it account for the even stronger "microcarpoid" element of *O. quadripetalus* in Labrador and Greenland, where *O. microcarpus* appears to be absent.

Conversely, when the two diploids are examined critically, examples may be found which indicate that these two populations, at some time, have exchanged genes. While by no means overly common, examples of *O. macro-*

[14] Hagerup found no tetraploids in the European material of *O. microcarpus*. In the case of *O. macrocarpus*, we feel rather certain of our conclusion. Since this species is the standard commercial cranberry, it has been selected from the wild and extensively cultivated and bred in North America for many years. Naturally, the plants brought into cultivation were selected for their vigor, fruitfulness, and size of fruit, characteristics which, in the Vaccinieae, we have learned to associate with polyploidy. No single commercial variety of *O. macrocarpus* has yet been found to be other than diploid.

carpus are known with "microcarpoid" pedicellary bracts, others have atypical length-width ratios in the leaves, leaf apices which are "microcarpoid," or obtuse or mucronate calyx lobes. By the same token, *O. microcarpus* is not completely homogeneous; some individuals have atypical length-width ratios in the leaves, some have blunter leaf apices than normal, and some have mucronate calyx lobes; and one plant was found with an almost "macrocarpoid" type of inflorescence. Of course, it might be supposed that this is a residual condition in both; that is, a situation wherein the genetic segregation was not yet complete when the original geographic disjunction took place. However, on the basis of the material available, the aberrancies in *O. microcarpus* seem to be most pronounced in the North American population. In my opinion, this points to a period of hybridization along the margins of the ranges of the diploids subsequent to a period of geographic isolation and stabilization, rather than a condition of residual genes. It would have been during such a period when a population of allotetraploids could have arisen out of the hybrids. I therefore favor the hypothesis that *O. quadripetalus* is a segregative allotetraploid.

But no matter what may ultimately be found to be the method of origin of this tetraploid population, I should like to emphasize one point. It is not my opinion that *O. quadripetalus* is a species of recent origin, except in comparison to the age of the diploids. Its present wide distribution would indicate that it arose prior to the Pleistocene, at a time when the continental masses of Europe, Asia, and North America were more closely connected. Furthermore, it seems likely that this tetraploid arose on what is now the North American segment of Holarctica, migrating from there into Europe by way of northern Asia. It would therefore seem logical to suppose that the primary divergence of the diploids must have been in progress in what is now North America at an even earlier date, probably not later than the mid-Tertiary.

SUMMARY

Recent work indicates that the cranberries, a group of the Vaccinieae commonly recognized as the genus *Oxycoccus*, occur as diploids, tetraploids, and hexaploids. Today, the diploids exist as two geographically disjunct populations, on the whole easily separable by a series of well-marked morphological characters. The southern diploid species, *O. macrocarpus*, limited to North America, appears to be the basic form, and *O. microcarpus* the derivative which, since, has achieved a wide distribution in high, northern latitudes, being in North America, Asia, and Europe.

A third population, essentially circumboreal in distribution, is known to be present; it is tetraploid and combines the characters of the two diploids. Although considerably segregative—so much so that various species, varieties and forms have been previously described from within it—there is no

evidence that any of the segregate types, even as aggregate groups, have achieved sufficient geographical or ecological disjunction to be worthy of more than subspecific recognition. Therefore, it seems best to treat this tetraploid population as a single species—*O. quadripetalus*. Although there is evidence that certain of these groups have attained a degree of differentiation as a result of selection, it is doubtful whether more than a simplified subspecific nomenclature would be of value, and even this could not be applied in any really critical manner. A more detailed analysis—possibly resulting in a complex and cumbersome nomenclature—should not be undertaken until considerably more material and information is available concerning this population from its entire distribution.

Although hexaploid material is known from Europe, it seems not yet to have been given satisfactory nomenclatural treatment. So far, only isolated, sterile, hexaploid plants are known. Since they appear to have been derived directly out of the tetraploid and, on the basis of those studied, are distinguishable from it primarily by their larger size, there seems to be no logical reason—should the need arise—why these forms could not be nomenclaturally appended to *O. quadripetalus*. However, as was pointed out, there is ample evidence from *Vaccinium* that hexaploids in the Vaccinieae can be completely fertile. Should future work in those areas in Europe, Asia, and North America where hexaploids are known, or suspected, reveal the presence of fertile plants in the process of building up an autonomous population, there would then be reason for the recognition of such hexaploid material as a valid species.

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BULLETIN OF THE TORREY BOTANICAL CLUB
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OCCURRENCE AND DISTRIBUTION OF THIAMINE, RIBOFLAVIN, AND NIACIN IN AVENA SEEDLINGS[1]

ILDA MCVEIGH

Certain of the B vitamins have been found to increase during the early stages of germination of cereal grains (Burkholder and McVeigh 1942). The object of this study is to correlate the changes in vitamin content of *Avena* seedlings at various stages in germination with changes in dry weight; also to determine the content and distribution of these vitamins in the organs of the seedling at successive stages in germination. The study was restricted to thiamine, riboflavin, and niacin, since they are known to function as parts of enzyme systems, and are therefore likely to be causally associated with cell division and enlargement.

Because the young *Avena* plant is so often employed as the test organism for growth hormones, it has been the subject of many investigations. Its cellular development, particularly that of the coleoptile, is now well understood (Avery, Burkholder & Creighton 1937). A study of the presence and distribution of physiologically active substances within the developing plant has been made in an attempt to determine the effect of these substances on the development of the plant.

According to Avery, Burkholder and Creighton (1937) cell division in the embryonic *Avena* coleoptile starts within 24 hours after the seeds are placed under suitable conditions for germination, and continues with appreciable though diminishing intensity until the coleoptile is about 10 mm. long, or until about the third day of germination. As long as cell division persists, it goes on with equal intensity throughout the coleoptile. Further growth is due almost entirely to elongation of cells.

Thimann (1934) reports that the amount of auxin in the *Avena* coleoptile decreases steadily with distance from the tip. The concentration of auxin per unit length of the root tip is somewhat less than in the coleoptile tip, but is about the same in the root base as in the coleoptile base. The decrease with distance from the tip is comparable in the two organs, and indicates, according to this author, that the auxin is produced in the tip. The concentration of the auxin within the first leaf is ten times that of the coleoptile tip (Thimann and Skoog 1940).

[1] This study was made at Connecticut College for Women, New London, Connecticut. The author expresses appreciation to Dr. George S. Avery, Jr., for making possible this investigation and for helpful criticism during the preparation of the manuscript.

The endosperms of germinating seedlings of *Avena* contain no ascorbic acid (Clark 1937). The coleoptiles of the same seedlings contain 0.54 mg./gm. fresh weight. Ascorbic acid is formed in the coleoptile, accumulating in the tip more than in other parts. This may mean that synthesis occurs at the tip, as is true of auxin. The precursor of ascorbic acid is probably moved from the seed and made active at the tip of the coleoptile.

Peptidase determinations were made on segments of *Avena* coleoptiles of various ages by Avery and Linderstrøm-Lang (1940). Peptidase activity, reduced weight, and cell number, when measured per segment, were found to decrease in progressively older coleoptiles. For any given coleoptile of 4 mm. or more in length, enzyme activity per unit weight of tissue or per cell is consistently greater at the tip.

A considerable amount of evidence concerning the occurrence and distribution of physiologically active substances in the *Avena* seedling, particularly in the coleoptile, has accumulated. Also there is a considerable amount of knowledge concerning the cellular behavior in these seedlings. The exact relationship between these physiologically active substances and cell division, cell enlargement, and differentiation remains to be determined.

MATERIALS AND METHODS

Victory oats (*Avena sativa* var. "Victory," obtained from Mr. Marshall Rumsey, Batavia, N. Y.) were grown on moist filter paper in large Petri dishes or culture dishes. These were placed in a dark room where temperature and relative humidity were maintained at 25° C and 88–90 per cent. One set of seedlings was exposed to greenhouse conditions during the day.

At daily intervals whole seedlings were harvested, counted, and dried in an oven at 70° C for 12 hours. Other plants were harvested, counted, and each divided into four parts: (1) endosperm, (2) coleoptile, (3) leaves, and (4) root system plus the first internode. After harvest separate organs were desiccated in the same manner as the entire seedlings. All material was preserved in the dry state over calcium chloride in a desiccator until the assays could be made. The material was ground fine in a glass mortar and extracts were made. For entire seedlings and the endosperms of various stages, 0.5 gm. samples were used. Each sample was autoclaved in 20 ml. of 1 N H_2SO_4 and the final volume of each extract was 30 ml. Glass-distilled water was used throughout, and the glassware was cleaned with chromic acid or calgonite solution, washed thoroughly, and rinsed in distilled water. The work was performed with the aid of filtered red light until the riboflavin determinations were completed.

Thiamine activity was determined by the *Phycomyces* assay method, as described by Bonner and Erickson (1938). Riboflavin and niacin were determined by the microbiological tests involving the use of *Lactobacillus casei*

and *Lactobacillus arabinosus* as described by Snell and Strong (1939) and Snell and Wright (1941). Thiamine tests were made in triplicate at one concentration level. Duplicate tests at each of four concentration levels were made for riboflavin and niacin. The amounts of extracts to be used were determined by preliminary trials and appropriate aliquots were selected, so that growth of the indicator organism would fall within a suitable range of response. The data are expressed as micrograms per gram of dry matter.

RESULTS

Seeds and Seedlings. Entire embryos were removed from one-day-old seedlings and the embryos and endosperms assayed separately. The ratio of the dry weight of the endosperm to the embryo is approximately one to fifteen. Assays revealed that the embryos at this stage of development contain, per gram dry weight, approximately 8 times the amount of thiamine, 7 times the amount of riboflavin, and 12 times the amount of niacin found in the endosperm. Per seedling, the embryo contains approximately 50 per cent as much thiamine and riboflavin and 80 per cent as much niacin as the endosperm.

To determine whether the high content of vitamins in the one-day-old embryos was due to a release or change of precursor into active form, brought about by soaking, unsoaked seeds and seeds from which the embryo had been removed were assayed. In removing the embryos from the dry seeds some portions of the endosperm were scraped away. Assays indicated that there is little or no change in the distribution of thiamine, riboflavin, and niacin in the embryo and endosperm during the first day of germination. A comparison of the assays of entire unsoaked seeds with one-day-old seedlings shows little if any change in thiamine content, approximately 10 per cent increase in riboflavin, and about 30 per cent increase in niacin.

The average dry weight of the seedlings decreased from about 23 mg. to 14.5 mg. during the 7 days' growth in the dark (fig. 1). Per dry weight the thiamine showed a slight increase, but this was only an apparent increase, since the dry weight decreases during germination. When the thiamine content is calculated per seedling it is evident that there is little if any change. The riboflavin and niacin reached the maximum value after five days' growth in the dark. Riboflavin increases approximately 13-fold per unit dry weight but only 8-fold per seedling. The most rapid increase in riboflavin occurs from the second to the fifth days. The apparent increase in niacin calculated per dry weight is nearly 9-fold but the actual increase is about 5.5 times. Niacin increases most rapidly during the first three days of germination.

A set of seedlings grown simultaneously with those just discussed, but exposed to greenhouse conditions during the day, was assayed after five

days' growth. The results agree with those obtained from plants of the same age grown in the dark, except that the riboflavin values are much lower.

Endosperm. During the seven-day period of growth the dry weight of the endosperm decreased from approximately 20 mg. to 2 mg. When calcu-

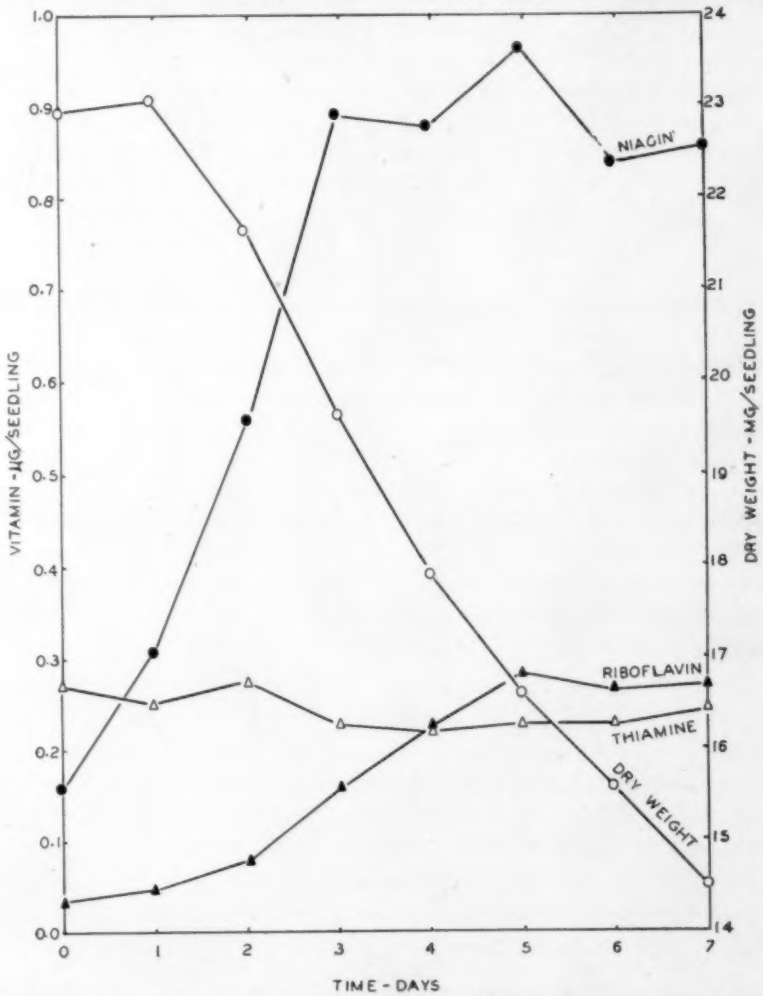
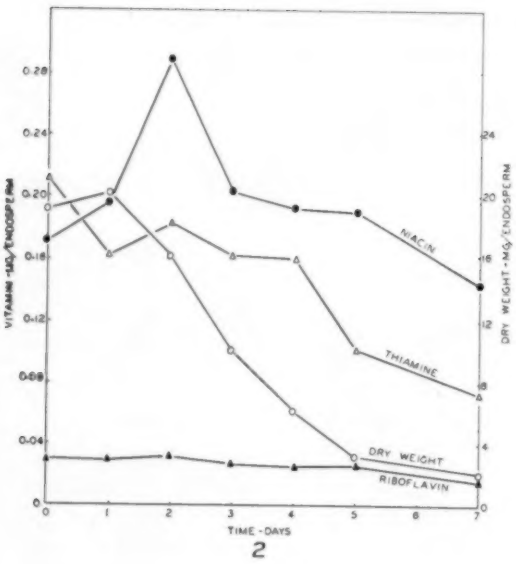
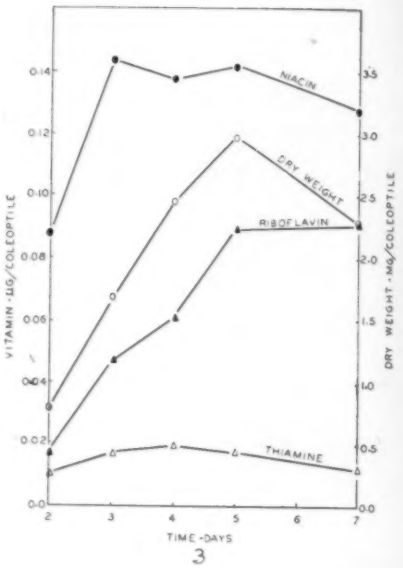
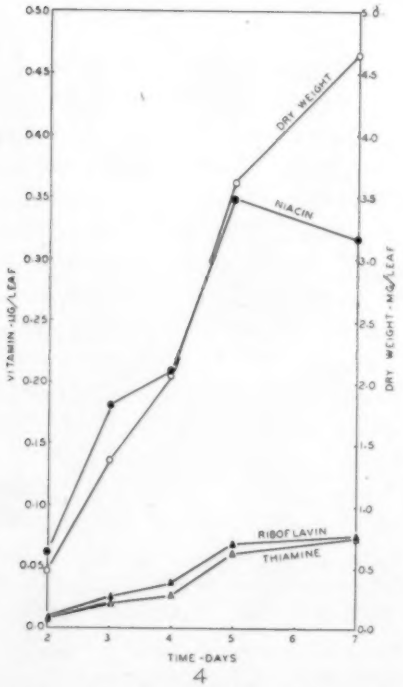


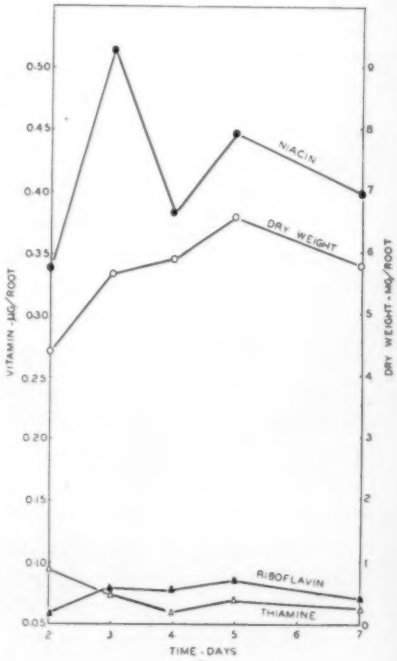
FIG. 1. Thiamine, niacin, riboflavin, and dry weight per seed and seedling of *Avena* grown for different lengths of time on moist filter paper in Petri dishes. Per seedling the riboflavin and niacin content increase during germination while the thiamine content remains nearly constant.

lated per gram dry weight, the three vitamins show considerable increases, but actually the stores of vitamins as well as food materials in the endosperm are depleted as the embryo develops (fig. 2). The riboflavin content of the









Thiamine, niacin, riboflavin, and dry weight of organs of *Avena* seedlings at successive stages in germination. FIG. 2. Endosperm. FIG. 3. Coleoptile. FIG. 4. Leaf. FIG. 5. Root.

endosperm is very low and changes very little during the 7 days' growth period; the greatest decrease occurs between the fifth and seventh days. The thiamine content falls off most rapidly between the fourth and seventh days. The niacin content increases rapidly during the first two days and then decreases at about the same rate. This initial increase is probably the result of the change of some precursor into an active form.

Coleoptile. The dry weight of the coleoptile increases from the second to the fifth day and then decreases (fig. 3). Per coleoptile thiamine increases slightly from the second to the fourth day and then decreases slowly. The riboflavin content per organ more or less parallels the change in dry weight during the first five days. The greatest increase in niacin occurs during the first three days.

Leaf. From the second to the seventh day the thiamine, riboflavin, and niacin content per leaf increases approximately 9-fold, 10-fold, and 7-fold respectively (fig. 4). During this period the dry weight increases about 10 times. Since the dry weight is increasing as fast or faster than the vitamins they appear to change little or decrease if calculated per unit dry weight.

Roots. The data for the roots over three days old are of questionable validity. Roots grown on moist filter paper are developing under very abnormal conditions and after about three days become so entangled in the paper that it is difficult to separate them. Riboflavin and niacin increase slightly per root during the early period of growth while the thiamine content decreases (fig. 5).

DISCUSSION

Since thiamine, riboflavin, and niacin function as parts of respiratory enzyme systems, it is not surprising that embryos contain rather large stores of them. Their role is one of making available to the young plant building materials stored in the endosperm. Ward (1943) has investigated the distribution of thiamine in the wheat grain. He reports that the cotyledon and epithelial layers are the centers of high thiamine concentration within the wheat kernel. One might expect the same distribution within the *Avena* seed.

As the seedling grows the vitamins are constantly being used. The assays do not indicate the total amounts of vitamins produced within the seedling but rather the excess of production over the amounts used.

The data suggest the leaves of *Avena* as centers of vitamin synthesis rather than the roots. During the growth period under consideration the leaves increase in each of the three vitamins. Assays of plants grown in soil under normal conditions should be made to supplement these studies.

SUMMARY

Avena seedlings, growing in the dark, make significant increases in riboflavin and niacin during the first five days of germination. There is little or no change in the thiamine content.

Approximately 50 per cent as much thiamine and riboflavin and 80 per cent as much niacin are contained in the embryo of an *Avena* seed as in the endosperm: the dry weight of the embryo is about 1/15 that of the endosperm.

With the decrease in dry weight of the endosperm during germination, thiamine and niacin per organ decrease also; the riboflavin content remains low. The leaf shows absolute increases in these three vitamins during the five-day period. The coleoptile increases in riboflavin throughout its period of growth, although its niacin content increases only during the first three days. The thiamine content of the coleoptile shows little or no change during germination.

OSBORN BOTANICAL LABORATORY, YALE UNIVERSITY
NEW HAVEN, CONNECTICUT

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